Early prediction of coagulopathy in acquired bleeding disorders - Revisited using Rotational thromboelastometry

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Experience in a tertiary hospital in South India

A dissertation submitted in partial fulfillment of M.D. Immuno Haematology and Blood Transfusion (Branch XXI) Examination of the Tamil Nadu Dr M.G.R. UNIVERSITY, CHENNAI to be held in 2015.

Acknowledgement

First of all I thank God Almighty with whom everything is possible.

I would like to take this opportunity to express my deep sense of gratitude and respect towards my teacher and guide Dr. Sukesh Chandran Nair, Professor, for his inspiration, invaluable guidance, constant encouragement, and expert suggestions.

I am thankful to Dr. Joy Mammen, Professor and Dr Rajesh Kannangai, Professor for their advice in statistical analysis of this study.

I am deeply indebted to Dr Anup Joseph Devasia, Senior PG Registrar, for his constant encouragement, valuable suggestions and healthy criticisms.

I sincerely express my gratitude to my dearest colleague Dr. Tulasi Geevar and Dr Vishnu for all the help, consideration and cooperation given to me during this study period.

My thanks are due to all the staffs of Department of Transfusion Medicine and Immunohaematology, especially Miss.Remya V, for the generous help they have provided throughout the duration of my course. I thank all others who have assisted me in some form or the other in the preparation of this work.

Salutation to my dearest mother, husband, brother, sister and my beloved daughter who were a constant inspiration at every stage of this work.

Last but not the least; I am grateful to all those patients who were the subjects for this study, without whose co-operation this work would not have been possible.

Certificate

This is to certify that the dissertation "Early prediction of coagulopathy in acquired bleeding disorders – Revisited using Rotational Thromboelastometry " is a bonafide work of Dr Soonam John towards the M.D. (Immuno Haematology and Blood Transfusion) Examination of the Tamil Nadu Dr M.G.R. University, Chennai to be held in 2015.

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Early prediction of coagulopathy in acquired bleeding disorders-Revisited using Rotational thromboelastometry

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CONTENTS

Title of the abstract: EARLY PREDICTION OF COAGULOPATHY IN ACQUIRED BLEEDING DISORDERS – REVISITED USING THROMBOELASTOMETRY

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Objectives

This study was done to establish a correlation between rotational thromboelastometryparameters and standard coagulation profile in the context of acquired bleeding disorders.

Background

Acquired bleeding disorders are a major cause of mortality, both in the developed and developing countries. An acute haemorrhage should be managed immediately with blood products, factor concentrates or antifibrinolytics. Investigations to detect coagulopathies typically include baseline screening tests like prothrombin time, activated partial thromboplastin time, platelet count and fibrinogen level. These tests have a long turnaround time which frequently lead to a blinded approach towards blood product support leading to under or over transfusion. In contrast, rotational thromboelastometry (ROTEM) which assesses haemostasis from the start of clot formation to fibrinolysis gives earliest results within ten minutes.

AIMS AND OBJECTIVES

To compare the diagnostic ability of Rotational Thromboelastometry (ROTEM) with the standard coagulation profile to detect coagulation defects in acquired bleeding disorders.

Methods

A total of 138 subjects - 70 patients who presented with acquired bleeding disordersand 68 subjects diagnosed to be normal on the basis of a complete coagulation work up were included as the cases and controlsrespectively. All samples were subjected to standard coagulation profile by automated methods and ROTEM analysis which included Clotting Time, Clot Formation Time, Alpha Angle, Maximum Clot Firmness and Maximum Lysis.

Results

The Maximum Clot Firmness shows a very good correlation with serum fibrinogen levels (k value - 0.807; $p<0.000$; Sensitivity - 88%; Specificity - 92%), and good correlation with platelet count (k value - 0.793; $p< 0.000$; Sensitivity - 86%, Specificity-92%), whereas Clot Formation Time showed moderate correlation with activated partial thromboplastin time, platelet count and fibrinogen levels.Alpha angle had a moderate correlation with platelet count and fibrinogen.Clotting time had a poor correlation with prothrombin time and activated partial thromboplastin time.

Discussion and Conclusion

The achievement of haemostasis is a crucial factor for determining patient outcomes in acquired bleeding disorders. The gold standard test to diagnose coagulopathy is the standard coagulation profile. Rotational thromboelastometry correlates well with standard coagulation parameters. This test which is performed on whole blood showed interpretable results within 10 minutes,

whereas standard coagulation profile required an average of 45minutes. In view of the good correlation to the standard coagulation profile, it appears that Rotational Thromboelastometryresults can be safely used to implement early transfusion therapy for haemorrhage.

Keywords: coagulopathy, rotational thromboelastometry, maximum clot firmness, alpha angle, clot formation time,

INTRODUCTION

Bleeding disorders comprise a varied group of diseases due to the malfunction of blood vessels, coagulation proteins and platelets. The clinical spectrum varies from a mild bleeding diathesis to severe hemorrhage which can lead to death. It can be due to either quantitative or qualitative abnormalities of coagulation proteins or platelets. Mild bleeding symptoms like easy bruising, epistaxis, are more common than severe life threatening hemorrhage. The symptoms may sometimes reveal an underlying malignant bone marrow pathology.

Bleeding-history assessment tools can be used to assess the patient. A high bleeding score indicates a significant bleeding disorder but there can be an overlap of scores for individuals with mild bleeding disorder and without any bleeding symptoms.(1) The bleeding histories are subjective and therefore significant symptoms may sometimes be ignored and considered as normal while negligible or minor symptoms be given undue consideration.

The bleeding disorders are broadly classified into inherited bleeding disorders and acquired bleeding disorders. Inherited bleeding disorders are diseases due to hereditary abnormalities of proteins or platelets which are necessary for blood clotting. The most common causes include Hemophilia A which is due to deficiency of coagulation factor VIII, von Willebrand disease which is caused by a defect or deficiency of von Willebrand factor and Hemophilia B or the Christmas disease, due to the deficiency of factor IX. Among the platelet disorders include Bernard Soulier Syndrome and Glanzmann Thrombasthenia.

Acquired bleeding disorders occur due to depletion, reduced synthesis, or inhibition of platelets and coagulation factors. The causes include disseminated intravascular coagulation, Vitamin K deficiency or antagonists, acquired inhibitors to coagulation factors, liver transplantation, renal causes, use of anticoagulant and traumatic bleeding. Disseminated intravascular coagulation can occur in various clinical conditions like septicemia, acute promyelocytic leukemia, abruption placenta, trauma. The most common condition resulting from a coagulation factor inhibitor is acquired Hemophilia A. The acquired platelet function disorders include drug usage,(Aspirin, Clopidogrel, Prasugrel) food additives, myeloproliferative neoplasm and Paroxysmal Nocturnal Hemoglobinuria.

Overview of hemostasis

The hemorrhage can be due to interference with the platelet plug formation, interference with blood coagulation proteins, or interference with both. The coagulation occurs by two pathways which includes the primary extrinsic pathway and the contactor intrinsic pathway (accessory pathway). Injury to the vessel wall results in exposure of tissue factor to factor VIIa which then forms the extrinsic tenase complex. The factors IX and X converts to factors IXa and Xa ultimately forming the intrinsic tenase complex and prothrombinase complex.The collective function of intrinsic, extrinsic tenase and the prothrombinase complexes results in thrombin generation. The thrombin acts as a procoagulant and anticoagulant. The thrombin splits fibrinogen into fibrinopeptides. The thrombin also activates factor XIII to form a cross-linked fibrin clot. (Figure 1)

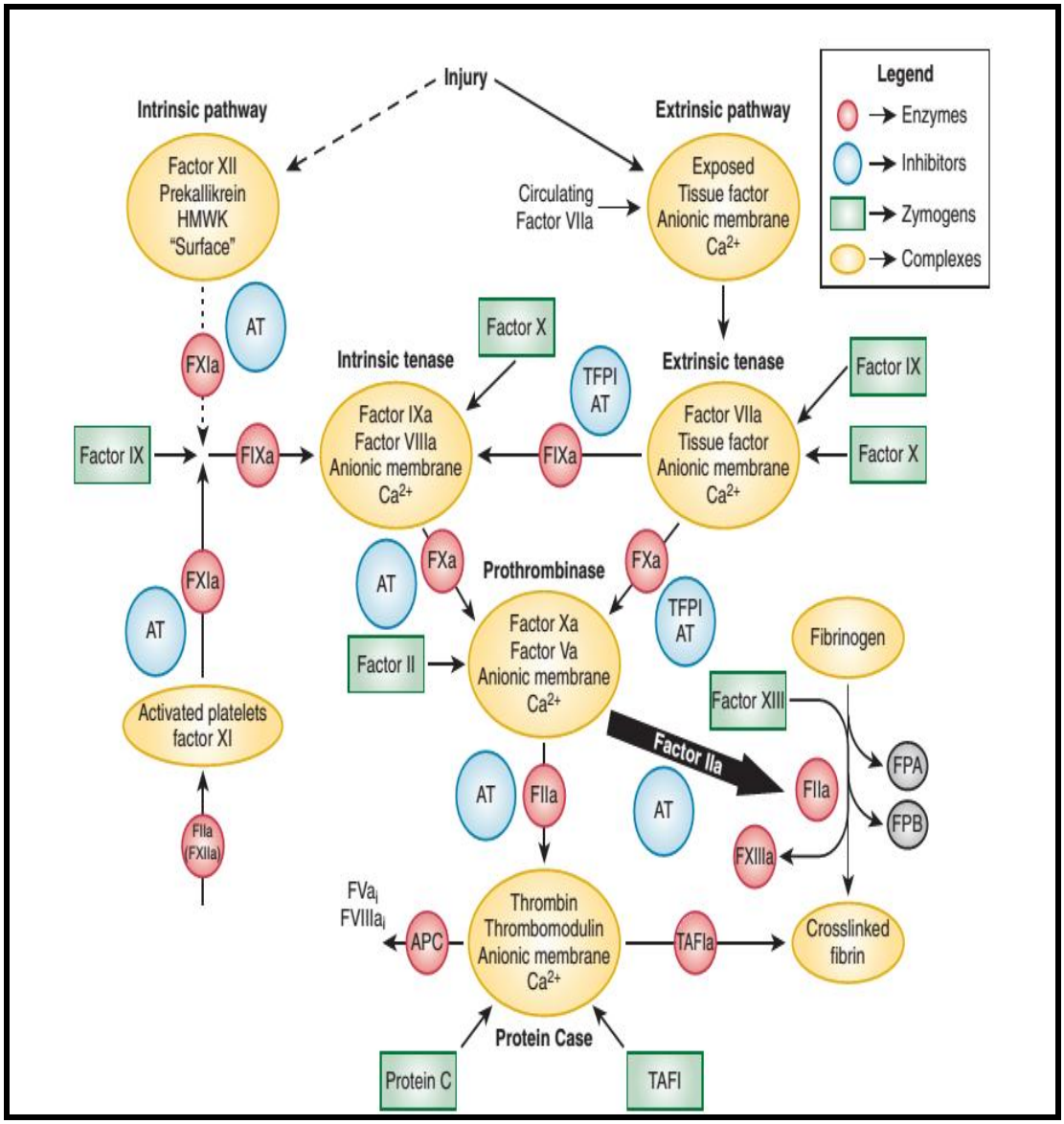

Figure 1- The coagulation pathway

Acquired bleeding disorders (ABD) are commonly occurs due to depletion, reduced synthesis, inhibition of coagulation factors and platelets. The clinical picture ranges from mild bruises to life-threatening hemorrhage. The location, frequency, severity of bleeding depends on the cause of bleeding disorder .A complete medical, surgical, family, social, and medication history is necessary in understanding the etiology. The basic laboratory test prothrombin time, activated

partial thromboplastin time, serum fibrinogen, platelet count, help in exposing the cause of bleeding. Management depends on the history and laboratory tests. The treatment decision includes administration of vitamin K, blood, plasma, cryoprecipitate or platelets transfusion and blood derivatives like factor concentrates.

Acquired bleeding disorders are the most frequent causes of hemorrhage encountered in the practice of hematology. The various type of disorders include drug induced hemorrhage, acquired factor deficiencies, disseminated intravascular coagulation, acquired coagulation factor abnormalities like acquired Hemophila A, acquired von Willebrands disease, immune thrombocytopenia, non–drug induced, acquired platelet function disorders, vitamin K deficiency, liver and renal disease, hypothyroidism, surgical bleeding, scurvy, hypothyroidism and Cushings syndrome. The most common cause is a drug-induced defect. (2)

An acute bleed should be managed immediately with blood products, factor concentrates antifibrinolytics before the results of coagulation test are available. The investigations for a bleeding problem includes prothrombin time, activated partial thromboplastin time, platelet count and fibrinogen level. Abnormalities, if detected, require further evaluation to determine if the cause is a fibrinogen disorder, platelet disorder or coagulation factors deficiencies.

Rotation thromboelastometry (ROTEM) are point-of-care devices that assess hemostasis from the start of clot formation to fibrinolysis. This system has four separate measurement channels which allow the independent tests like EXTEM, INTEM, APTEM AND HEPTEM and a computer for automatic analysis. This study was done to assess the efficiency of rotational thromboelastometry in comparison to the standard coagulation profile in assessing coagulopathy in acquired bleeding disorders.

REVIEW OF LITERATURE

History

In 1628, William Harvey described the circulatory system.(3) In 1825, the first successful human-to-human blood transfusion was reported by James Blundell, an obstetrician and physiologist at Guy's Hospital in London who transfused women with postpartum haemorrhage.(4) During World War I and II blood transfusion was given to bleeding soldiers to combat the war injuries.(5)

Ratnoff and Davies proposed the coagulation pathway, which was composed of a series of proteolytic reactions starting with factor XII and terminating in the formation of clot. (6) The prothrombin time was introduced by Dr Armand Quick in 1935 which was used to assess coagulopathy in Hemophilia and was later used in acquired bleeding disorders.(7) In 1936, Warner et al developed a two-stage prothrombin assay, based on the fact that plasma, serum factors and tissue factor are required for the conversion of prothrombin to thrombin. (8) The partial thromboplastin time was illustrated by Langdell and was used to assess Hemophila. The platelet count estimation was done with the help of automated platforms since the early 60's.(9)

Homeostasis

In the beginning of twentieth century, Paul Morawitz, a German physiologist put forward the classic theory of coagulation. He hypothesized that thromboplastin converts prothrombin to thrombin converting fibrinogen to the fibrin. These clotting factors were named as fibrinogen, prothrombin, thromboplastin, tissue factor membrane and calcium. The factors that were discovered later were assigned consecutive Roman numerals. The activated forms were depicted by lower case a .(6)

The hemostatic system or the coagulation cascade was based on the waterfall hypothesis of Ratnoff and Davies(9) and MacFarland,(10) who independently reported a series of proteolytic reactions beginning with factor XII activation and ceasing with the thrombin converting fibrinogen to form a thrombus. The shortcoming of this hypothesis was that factor XII deficiency was not associated with bleeding.(11) The cofactors for factor XII activation; prekallikrein and high- molecular-weight kininogen deficiencies also were not associated with hemorrhage.(12)

Over the decades these pathways have been modified The newer concept of coagulation represents a complex process of procoagulant and fibrinolytic actions which occur simultaneously.

The Vitamin K–dependent protein family

Vitamin K–dependent factors are produced in the liver. These factors act as either procoagulant or anticoagulant. These include factors II,VII, IX, X and the anticoagulants factors protein C, protein S, and protein Z. (13) Vitamin K is essential for the synthesis of these clotting factors which undergoes γ-carboxylation.(14) This alteration adds a negative charge to the molecules that enables proteins to interact with Calcium and surface of membrane. Vitamin K–dependent protein complexes are composed of a serine protease enzyme, a cofactor, and a negatively charged membrane surface. The four vitamin K–dependent complexes include the extrinsic tenase complex (FVIIa and tissue factor), the intrinsic tenase complex (FIXa–FVIIIa), the prothrombinase complex (FXa–FVa), and the anticoagulant protein Case complex (thrombin–thrombomodulin).

Cofactor proteins

1. Cell bound cofactors - Tissue factor and thrombomodulin.

2. Soluble plasma-derived procofactors - factor V, factor VIII and von Willebrand factor .(6)

Tissue factor

A cofactor for factorVIIa in the extrinsic tenase complex. The release of tissue factor to the circulation initiates the procoagulant pathway. (15)

Thrombomodulin

A protein expressed on the surface of vascular endothelial cells. It acts as a cofactor for the activation of protein C. (16) The formation of protein C by the thrombin–thrombomodulin complex causes inactivation of factor Va and factor VIIIa.(17) This complex has an antifibrinolytic action by activating the fibrinolysis inhibitor, thrombin activatable fibrinolysis inhibitor (18)

Factor VIII

The factor VIII also known as the Antihemophilic factor circulates in plasma in complex with von Willebrand factor(19) The von Willebrand Factor–free factor VIIIa forms a complex with factor IXa, Calcium and platelet membrane resulting in the formation of intrinsic tenase complex.

The Intrinsic Accessory Pathway Proteins

The proteins of the intrinsic or accessory pathway includes factor XII, prekallikrein, and high molecular-weight kininogen. (20) The function of the accessory pathway is not fully

understood.(21) This pathway play an important role in disseminated intravascular coagulation (DIC) associated with the systemic inflammatory response syndrome and also in cardiopulmonary bypass where there is contact between blood and synthetic surfaces.(22) The factor XI is an intersection point for the two pathways. Individuals with factor XI deficiency have a inconsistent bleeding phenotype proving that there is a role for factor XI in hemostasis. (23) The propagation phase of thrombin generation is carried out by FXI a.(24)

The clot formation inhibitors in the blood include antithrombin, heparin cofactor II, tissue factor pathway inhibitor and protein C inhibitor.

The integrity of vasculature is necessary for normal hemostasis. The endothelium that lines the interior of blood vessels, plays an important role by providing a structural barrier to blood regulates blood pressure and deposits an intricate basement membrane and extracellular matrix. Massive bleeding can occur due to abnormalities of the endothelial cells.(25) Studies by Ware and Seegers had identified that phospholipid is required for coagulation.(26) The damaged vascular tissue activated platelets produce phospholipids. The endothelial damage release von Willebrand factor that causes platelet aggregation and adhesion to the sub endothelium leading to thrombus formation.

Platelets are small, cell fragments, derived from megakaryocytes contribute to procoagulant events and fibrinolytic process. The platelets contain secretory granules: α-granules, lysosomes, and dense granules. The activated platelets adhere to each other, endothelial cells, leukocytes, and components of the subendothelial matrix.(6)The phosphatidyl serine surface platelets are present a procoagulant surface to the circulation. The activated platelets express factor Va

binding sites (27) Factor Xa bind to effector cell protease receptor-1 molecules which are present on activated platelets.(28)

The presence of receptors on the platelet surface thrombin, adenosine diphosphate, and thromboxane A2 to channel other platelets into the hemostatic. The close contact between platelets promote stabilization of the hemostatic plug (29)

Clot proteins

Fibrinogen helps in hemostasis to prevent hemorrhage. It supports inter platelet aggregation. Fibrinogen controls thrombin activity to form a fibrin clot. Fibrinogen consists of polypeptide chains with the NH2 termini cross linked to each other. Fibrinogen increases during inflammation as it is an acute phase reactant.(30)

Fibrinolysis or the removal of blood clots includes primary fibrinolysis and secondary fibrinolysis. The primary fibrinolysis is a normal process while secondary fibrinolysis is the due to a medicine or a medical disorder. Plasminogen is the precursor of the plasmin, which is the primary catalyst of fibrin degradation.(31) The activation of plasminogen occur through three pathways: (a) the intrinsic activator system (b) the extrinsic activators (c)an activator system involving fibrinolytic drugs. The plasmin degrades fibrin producing degradation products.

Initiation

The injury to the vessels results in a triggered response resulting in platelet and fibrin deposition. The coagulation enzymes anchors, assembles, and express on the activated platelet membrane.(32) As vascular damage progress, more activated platelets gets anchored which allows the assembly of more coagulation enzymes leading to increased fibrin formation. When

the vascular system is perturbed the extrinsic tenase complex which is the tissue factor–factor VIIa and the calcium complex , comes into play.(33) The extrinsic tenase complex activates low levels factor X and factor IX to Xa and factor IXa.(34) The time interval in which factor Xa generates thrombin is called as the initiation phase of blood coagulation.(35) During the initiation phase, circulating blood cells and procoagulant proteins are activated, and a initial fibrin network is formed.(36)

Amplification

Thrombin also activates factor XI to Xia , initiating the accessory pathway that promotes factor IX activation.(37) The aggregated platelets and fibrin due to the thrombin formation are the major constituents of the initial vascular plug formation. The cofactor factor VIIIa combines on activated platelets with the factor IXa to form the intrinsic tenase complex which activates factor X. (38) Factor Xa formed from factor X combines with factor Va on the platelet membrane to form the prothrombinase complex which generates thrombin. (24) The prothrombinase and the intrinsic tenase complex are protected from inhibition by antithrombin.

Propagation

The aggregate of activated platelets will cause an increase in intrinsic tenase and prothrombinase complex formation on their surfaces with the help of specific platelet receptors. These platelet-bound complexes cause the propagation stage of the reaction resulting in the production of enormous quantities of thrombin. (39) This stage is independent of tissue factor and is continuous. Prothrombinase complex formation requires generation of factor Xa. It is formed through the intrinsic tenase and the extrinsic tenase complexes. The concentration of factor Xa is the rate-limiting factor for formation of prothrombinase complex.(40)

The coagulation mechanism is susceptible to factor V and platelets and is affected in case of congenital deficiencies, thrombocytopenia, abnormal platelet function or pharmacologic interventions.(41) Nearly 95% of thrombin is produced during the propagation phase after formation of fibrin clot.(42)

Termination

When blood flow stops due to the formation of a fibrin clot, the TFPI, antithrombin, heparin cofactor II, α2-macroglobulin, α1-antitrypsin, and protein C inhibitor, inhibit the various reactants as they dissociate from their respective complexes.(43) In the intact blood vessel surrounding the newly formed clot, procoagulant enzymes are rapidly reduced under normal circumstances. The thrombin, factor IXa, and factor Xa are repressed by the excess antithrombin molecules. The thrombin bind with thrombomodulin and is converted to an anticoagulant enzyme from a procoagulant state.(44) This complex activates protein C, which causes decrease of intrinsic factor tenase and prothrombinase. . These intravascular mechanisms function against occlusion of the blood vessel.

Elimination and Fibrinolysis

The hemostatic clot is composed of aggregated platelets and fibrin. There are some plasma proteins and blood cells which are trapped within the clot. Finally clot disintegration occurs with the help of plasmin which maintains hemostatic balance. The tissue plasminogen activator generates plasmin at the fibrin surface and governs fibrin homeostasis, and u-PA generates pericellular plasmin.(45) The t-PA and plasminogen bind to the fibrin surface which results in augmented plasminogen activation. Fibrinolysis is regulated primarily by PAI-1 (46) PAI-2, α2-antiplasmin, and TAFI. Antiplasmin is the primary inhibitor of plasmin. The α2-antiplasmin, bound covalently by factor XIIIa blocks plasmin binding and decrease plasminogen

activation.(47) The elimination phase begins with tissue repair by dissolving the fibrin–platelet clot generated earlier in hemostasis. The plasmin clears the fibrin clot and also removes damaged tissue. If a sufficient hemostatic plug is not formed to arrest blood flow, it can result in pathologic hemorrhage.

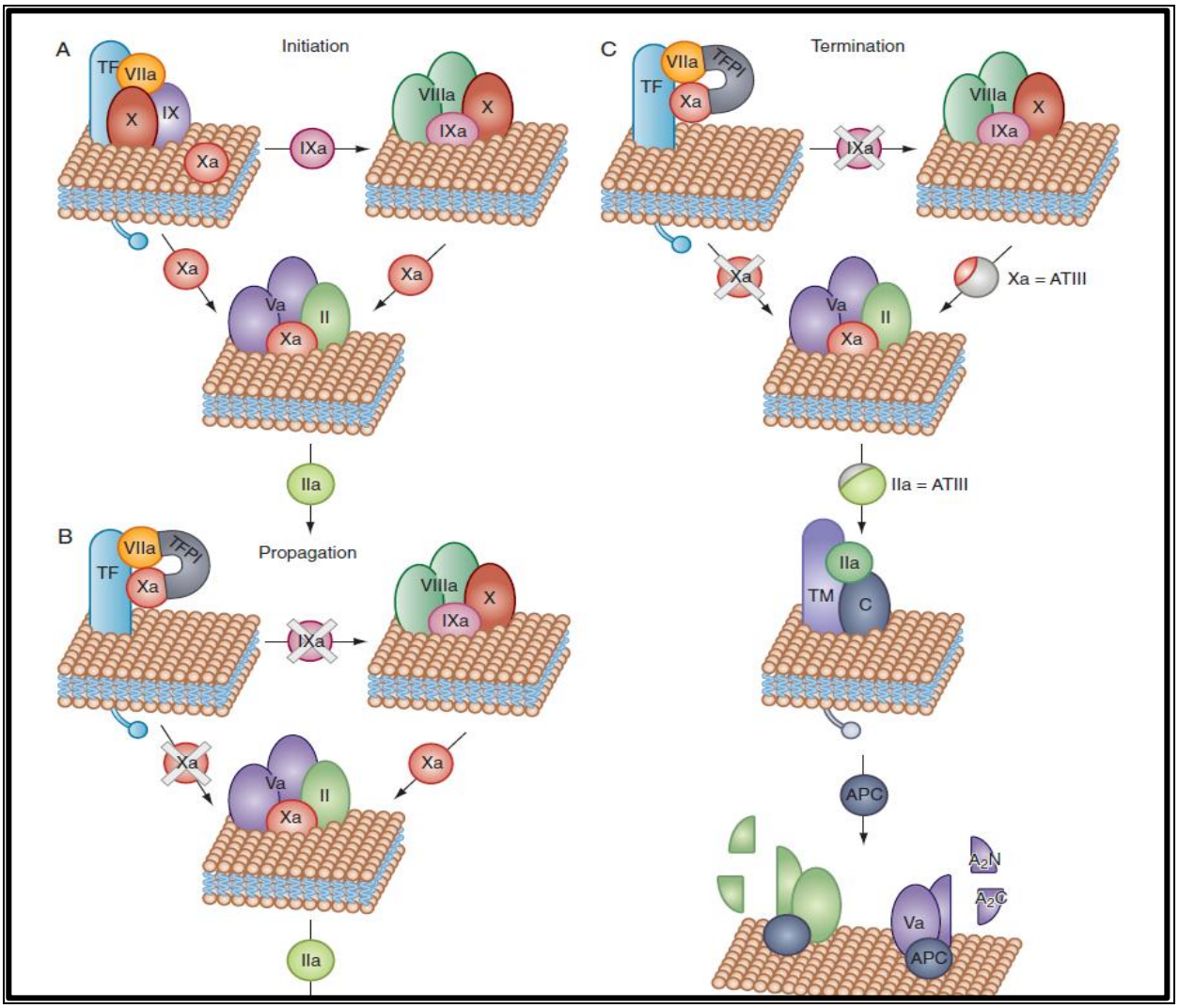

Figure 2: Regulation of coagulation cascade

Acquired bleeding disorders

Acquired bleeding disorders are commonly seen in the practice of hematology. The symptoms vary from mild bruises to life threatening hemorrhage. The clinical, family, social, and medication history site, frequency and the age of onset aids in the diagnosis of the type of disorder. These disorders can be due to consumption, reduced synthesis, inhibitors to either coagulation factors or platelets. The treatment depends on the cause of bleeding and includes blood component transfusion, factor concentrates administration of vitamin K and anti fibrinolytic agents.

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is one of the most common acquired bleeding disorder. Disseminated intravascular coagulation is the widespread activation of coagulation system,that results in the intravascular formation of fibrin leading to thrombotic occlusion of small blood vessels. Intravascular coagulation cause decreased blood supply to organs and, in combination with hemodynamic and metabolic derangements cause organ failure. Ongoing coagulation causes depletion of platelets and coagulation proteins which results in severe bleeding. Hemorrhage will be the presenting symptom in such cases and this can complicate decisions about treatment.(48) This event causes utilization of coagulation factors which can lead to bleeding disorder if the consumption is high. This condition is mainly associated with septicemia, malignancy, and fulminant liver disease. DIC can also occur in severe trauma, toxic poisonings, snake bite, placental abruption and amniotic fluid embolism.(49) The most important clinical conditions associated with DIC are listed in Table 1. The clinical feature are usually unpredictable. There can be a disseminated bleeding, like an ooze from multiple sites. Major

bleeding manifestations and intracranial bleeds are relatively rare. The occlusion in the vasculature of organs by microthrombi results in necrosis causing multiple organ failure.(50)

CLINICAL CONDITIONS THAT CAUSES DIC
Sepsis
Trauma , Burns or heat stroke
Malignancy – Solid tumours, AML M3
Obstetric conditions – Amniotic fluid embolism, Placental abruption, HELLP
Vascular abnormalities- Aortic aneurysms, Kasabach-Merritt syndrome
Allergic reactions
Immunological reactions

Table 1 - Causes of Disseminated Intravascular Coagulation

Pathogenesis

The endothelial cells and activated mononuclear cells generate pro inflammatory cytokines that cause release of tissue factor which starts coagulation. The suppression of anticoagulant action and inhibition of fibrinolysis causes intravascular fibrin deposition.

Clinical manifestations

Disseminated Intravascular Coagulation in Infectious Disease

Infections are the most common causes of DIC. Septicemia is the most common clinical condition associated with disseminated intravascular coagulation. Mostly all microorganisms can cause disseminated intravascular coagulation and bacterial infection is most frequently related to the development of this syndrome. The triggers of the activation of coagulation in septicemia patients are cell membrane components of the microorganism which includes lipopolysaccharide , bacterial exotoxins which induce a generalized inflammatory response causing release of cytokines.(51)

Newborns and post splenectomy patients are prone to DIC.(52) Hemorrhage can be further aggravated by thrombocytopenia. Bacterial infections are more commonly associated with the syndrome. Viruses , protozoa , and fungi can cause DIC.(53) In septic patients can present with thromboembolic disease or multiple organ dysfunction.(48)Patients may present with severe bleeding in some cases.(54) Purpura fulminans is lethal form of DIC which affects infants and children in which the skin over the extremities undergo hemorrhagic necrosis due to diffuse thrombi in small blood vessels . This disorder occur due to meningococcemia,streptococcal disease or staphylococcus.(55)

Disseminated Intravascular Coagulation in Trauma and Burns.

Traumatic hemorrhage resulting in trauma induced coagulopathy is associated with mortality in severely injured patients. The blood loss following severe injury can lead to disturbance of the blood coagulation system causing trauma induced coagulopathy (TIC) which is due to hypo perfusion of tissue and loss and severe tissue injury.(56) The severity of Disseminated Intravascular Coagulation depends on time gap between trauma and management. Provision of

prompt medical care can reduce the risk of DIC. The massive release of Tissue factor to the blood circulation, hemorrhagic shock, cytokines are possibly the factors that initiate DIC .TIC is due to dysfunction of inflammation, anticoagulation, and cellular systems. The underlying mechanisms of TIC have not been fully revealed. In addition dilutional coagulopathy, hypothermia, and acidosis also contributes to TIC. (56) Surgical excision of burn wounds causes severe bleeding leading to coagulopathy .(57)

Disseminated Intravascular Coagulation in Obstetric and Gynecological Complications

Postpartum hemorrhage (PPH) is significant problem in obstetrics and a leading cause of maternal deaths worldwide. The incidence of PPH is approximately 5%-20% of labors (58) with the highest rates in developing countries. Postpartum hemorrhage is associated with disseminated coagulopathy. The activation of coagulation system results in reduction of platelets and coagulation factors, aggravating bleeding due to consumption.(59)

The majority of cases due to obstetric pathology. The major obstetrical cause of postpartum hemorrhage is uterine atony which can lead to coagulation failure. (60) Placental abruption causes perinatal death and can lead to DIC. (16) Antenatal women with hypertension at highest risk. DIC can occur in such situations due to of enormous amounts of tissue factor in the blood circulation. The leakage of thromboplastin-like product from placenta which occurs in abruption leads to disseminated intravascular coagulation.(61)

In amniotic fluid embolism , the fluid enters the maternal circulation through tears in the chorio-amniotic membranes and uterine rupture. DIC is caused by the tissue factor present in amniotic fluid. The autopsy finding in patients who died of amniotic fluid embolism had

revealed fetal debris and the meconium, in the amniotic fluid causes thrombus formation and fibrinolysis, leading to the occlusion of the pulmonary arteries causing breathlessness, cyanosis, shock, acute corpulmonale, left ventricular dysfunction and convulsions. These symptoms are followed by severe bleeding in some patients. (62)

Disseminated Intravascular Coagulation in Malignancy

Patients with solid tumors are susceptible to DIC that can exacerbate hemorrhage. The risk factors include old age, stage of the disease, chemotherapy , infection, liver metastases .(63) In acute promyelocytic leukemia, increased fibrinolysis causes hemorrhage. Studies in mice model suggested that annexin II have a role in acute promyelocytic leukemia coagulopathy.(64) The blasts overexpress annexin II, a receptor for tissue plasminogen activator (tPA), and plasminogen, and increases plasmin generation. The brain and lungs are the most common sites affected which can lead to fatal hemorrhage.(65)

Solid tumor cells express procoagulant molecules like tissue factor , which forms a complex with factor VII and activate factors IX and X, an endopeptidase which activates factor X.(66) . Nearly 15% to 20% of acute lymphoblastic leukemia, present with disseminated intravascular coagulation.(63)

Disseminated Intravascular Coagulation With Vascular Disorders

Disseminated intravascular coagulation can be caused by vascular anomalies. Aortic aneurysms can cause localized platelet and fibrinogen consumption and can produce coagulation abnormalities and bleeding.(67) Kasabach and Merritt described bleeding in patients with giant cavernous hemangiomas, that cause consummation of platelets and fibrinogen.(68)

Disseminated Intravascular Coagulation With Toxic Reactions or Snake Bites

The venom of vipers and rattlesnakes, can produce a coagulopathy similar to that of DIC.(69) The venoms of these snakes contain enzymes that function by the following mechanism:

a) release fibrinopeptide A

b) activate prothrombin even in the absence of calcium

c) activate factors X and V

d) degrade fibrinogen

e) cause platelet aggregation

f) inhibit platelet aggregation

g) Damage endothelial cells and cause bleeding, tissue ischemia, and edema.

The victims of snake bites rarely have excessive hemorrhage.(6)

Liver disease and orthotopic liver transplantation

Low platelet counts is frequent in liver disease and the platelet counts range from 30 - 100× 10^9/L.(70) Platelet aggregation also occurs which causes prolonged bleeding time. (71) The increase of tissue plasminogen activator in plasma and decrease of the naturally-occurring inhibitors of plasmin results in hyperfibrinolysis (72)

Even in a scenario of deranged hemostasis , routine hemostasis tests fail to reveal the bleeding tendency in cirrhosis (73–75). The low procoagulant factors are balanced by decrease of

naturally occurring anticoagulants.(76) Plasma hyperfibrinolysis has been reported in patients with chronic liver disease (36)

In liver transplantation age, severity of liver disease, hemoglobin and plasma fibrinogen values determine the bleeding crisis. During the hepatectomy and the anhepatic phase, hemorrhage occurs due to decrease in clotting factors either due to surgical bleeding or the primary disease. The coagulopathy exacerbates on infusion of crystalloid, colloid, or blood products.(77) At the time of graft reperfusion, further depletion of all coagulation factors, antithrombotic factors occurs which further aggravate bleeding. (37)

Renal disease

Bleeding diathesis have been reported in patients with uremia especially when they undergo surgery and other invasive procedures. Life -threatening conditions like pericardial tamponade, intracranial bleeding, and gastrointestinal bleeding are also seen in these conditions.(78) The patients have a platelet dysfunction and an abnormal platelet–vessel wall interaction.(79) Various studies have shown that contact of fibrinogen to ADP-stimulated platelets is impeded and the interaction between vWF and the GPIIb-IIIa is disturbed.(80)

Vitamin K deficiency

Vitamin K deficiency is usually seen in newborns especially those with mal absorption or on broad-spectrum antibiotics treatment. Adults who have reduced stores of vitamin K or due to warfarin overdose, rodenticide poisoning (called super warfarins as they have the same mechanism of action like warfarin) are prone for bleeding diathesis.(81,82)

Vitamin C deficiency (Scurvy)

This is a rare condition especially in developed countries, when patient presents with perifollicular hemorrhages and gum bleeding (83). There are case reports of scurvy patients presenting with hemarthrosis.(84)

Massive transfusion

Massive transfusion is defined as 1) transfusion of more than 10 red cell concentrate units, which is nearly the total blood volume of an average adult patient, within 24 h 2) transfusion of more than four red cell concentrates in 1 h with expectation of continued need for blood product support 3) replacement of fifty percent of the total blood volume by blood products within 3 h.(85–87) The hemostatic defects in such patients have multifactorial pathogenesis causing early trauma induced coagulopathy leading to further transfusion support.(88) Coagulopathy can be aggravated by the infusion of crystalloids and blood products The transfusion of red cell units without additional clotting factors or platelets causes further impairment of hemostasis due to dilutional coagulopathy , thrombocytopenia ,acidosis , hypocalcaemia and hypothermia from refrigeration.(89,90)

Acquired coagulation factor inhibitors

These disorders can be due to immune or nonimmune causes. Antibodies are produced against the coagulation factors which causes hemorrhagic manifestations. Acquired Hemophila A is a rare bleeding disorder, autoimmune in origin with an incidence of 0.2 to 1.0 cases/million population per year.(91) The hemorrhages in patients with acquired hemophilia usually affect soft tissues. (92)The factor VIII autoantibodies rarely in pregnancy causing bleeding symptoms. (93) Acquired Hemophila B cases are very rare. A few cases has been reported so far. (94)

Acquired factor V inhibitor is another rare entity in the elderly, that presents with hemorrhagic manifestations. The probable causes for the inhibitor development include exposure to bovine thrombin, antibiotics, surgery and malignancy. (95) Acquired FX deficiency presents with variable symptoms, and there are various case studies showing association with amyloidosis.(96,97)

Hypothyroidism

There are case reports on association of hypothyroidism with acquired von Willebrand disease presenting with bleeding manifestations .The coagulation defects were corrected with the management of hypothyroidism.(98,99)

Cushing syndrome

Easy bruisability and menstrual disturbances are features of Cushing syndrome or treatment with systemic or topical glucocorticoids.(100)

Drug -induced platelet dysfunction

Aspirin, NSAIDs, other platelet function inhibitors are common causes of drug induced bleeding.(101,102)

Anticoagulation

Acute bleeding following anticoagulant therapy might be severe or even life-threatening and the frequency ranges from 2% to 13%. Drugs like Warfarin inhibits the action of vitamin K.(Figure 3) The management is the reversal of anti-coagulation depends on the situation, as per the international normalized ratios (INR), severity of bleeding and indication. The management

includes reversal of anticoagulant, vitamin K injection. In emergency situations fresh frozen plasma and coagulation factor concentrates can be used. (69)

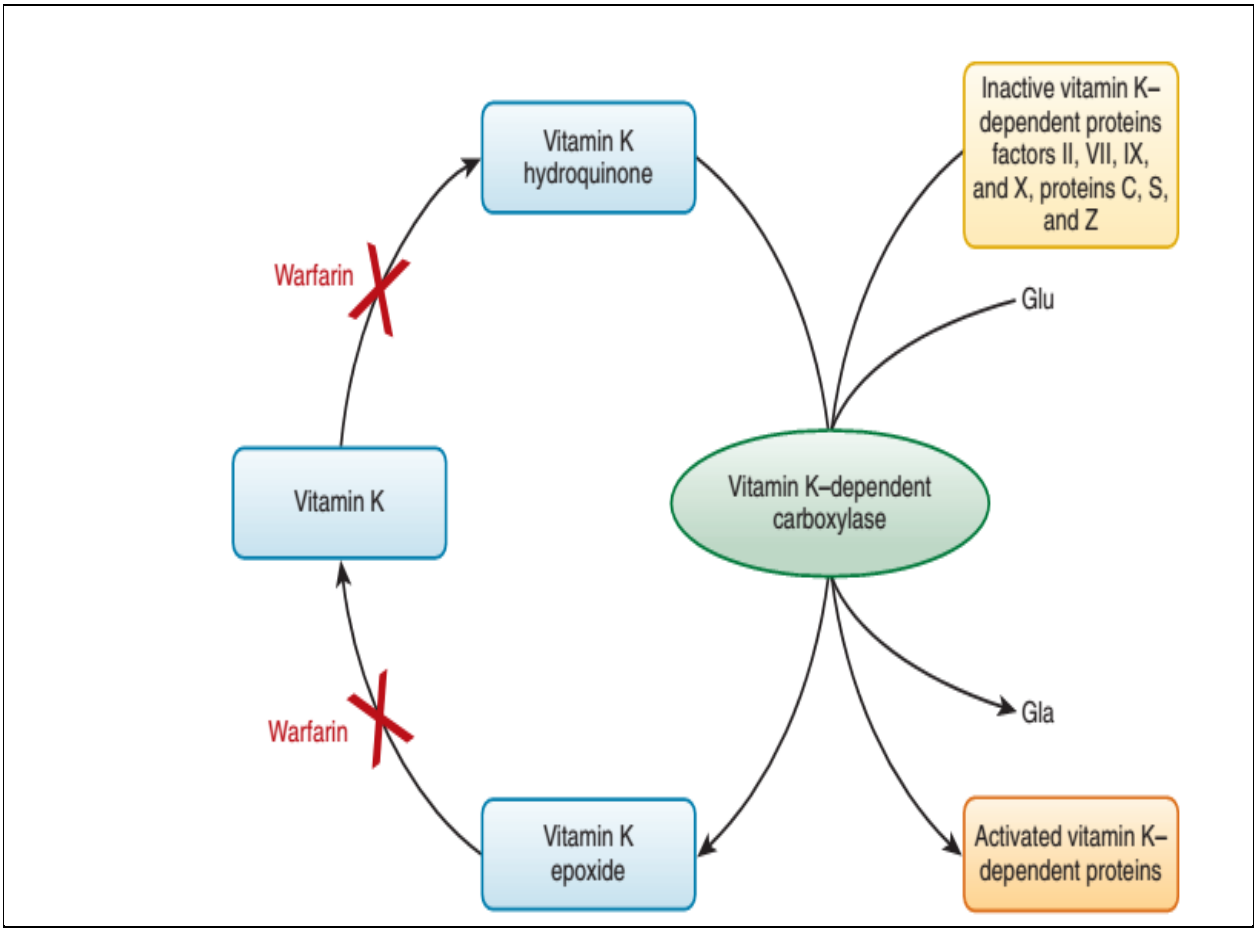

Figure 3 – Mechanism of action of Vitamin K antagonists.

LABORATORY ASSESSMENT

STANDARD COAGULATION PROFILE

The diagnosis of disseminated intravascular coagulation is based on clinical presentation and an altered standard coagulation profile. A rapidly decreasing platelet count is an important feature of DIC.(12) Due to consumption of coagulation factors , both PT and aPTT are prolonged. The

APTT can be very shortened in early acute DIC due to the presence of activated coagulation factors.(103) Fibrinogen depletion occurs in severe DIC but can be high or normal since it is an acute phase reactant.(104) In severe cases of DIC, microangiopathic hemolytic anemia may be seen .Disseminated intravascular coagulation can be diagnosed on the basis of the following:

a) An underlying cause like sepsis, trauma, burns.

b) Platelet count of less than 100,000 per cubic millimeter/ rapid decline in the platelet count.

c) Prolonged prothrombin time and the activated partial-thromboplastin time

d)The presence of fibrin-degradation product.

e) Depleted plasma fibrinogen. (48)

Low plasma fibrinogen levels of less than 50 mg/dL are associated with severe hemorrhage in DIC. Fragmented red blood cells or schistocytes will be seen in DIC cases. Toxic changes in leukocytes will be present in the smear of sepsis patients.(105)

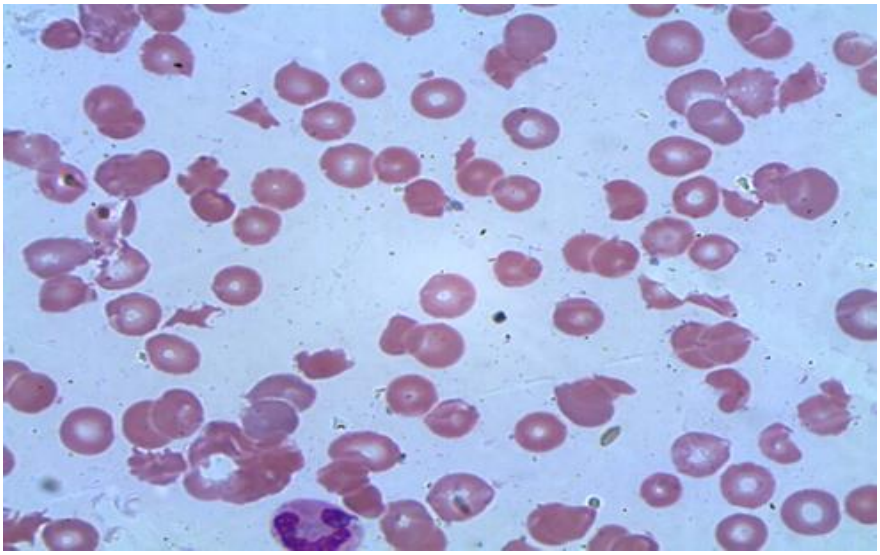

Figure 4- Peripheral smear showing schistocytes in DIC

LIMITATIONS OF STANDARD COAGULATION PROFILE

The PT and aPTT tests identify only partially if any coagulation defects are present. These tests also give a high number of false positive and false negative results.(106)In obstetrics pre-procedural coagulation screening is usually not recommended. A complete bleeding and medication history is considered more effective than doing coagulation profile. If there is a hemostatic impairement like placental abruption , coagulation profile is done.(107)

THROMBIN GENERATION TIME

Thrombin which acts as a procoagulant and anticoagulant is an important enzyme of the coagulation cascade. The thrombin generation is one of the prime steps in coagulation. The thrombin generation test is suitable for its assessment. This test detects both pro- and anticoagulant processes, so it can be applied for the detection of thrombosis and bleeding. Anticoagulant therapy can be monitored by this test. This assay can be used to assess bleeding severity , monitoring treatment and inhibitor by passing therapy in Hemophilia cases.(108)

THROMBOELASTOGRAPHY

Thrombelastography is a test which measures viscoelastic properties of blood clot. This test is used in trauma settings for managing acute coagulopathy and helps in taking decision regarding transfusion. This test can be used as a point of care test and can be performed by trained personnel. Moreover the technique requires standardization. Partial results which can be used for bleeding assessment is available in a short time, while whole test may require long time periods as other conventional tests. The clot strength and fibrinolysis can be assessed by thromboelastography. (Figure 4) Clot strength measure can determine whether there is

coagulopathy Thrombelastography can diagnose fibrinolysis and can be used to decide on anti-fibrinolytic drugs, cryoprecipitate and fibrinogen concentrate therapy. (109)

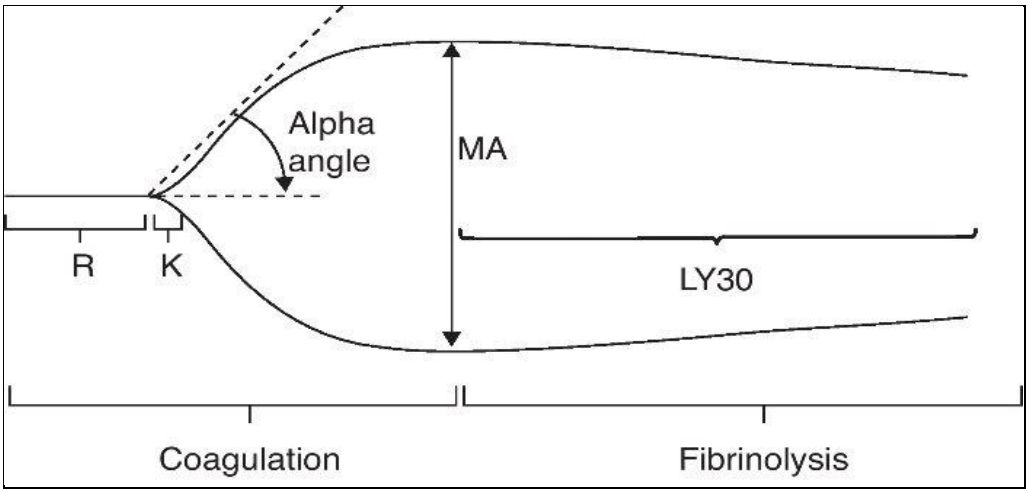

Figure 5- Normal thromboelastography

ROTATIONAL THROMBOELASTOMETRY

Background

Rotational thromboelastometry is a point of care (POC) viscoelastic tests of hemostasis which detects clot formation and dissolution. ROTEM is a variation of the TEG technology originally described by Hartert in1948. This test provide a visual evaluation of clot formation, propagation, stabilization and lysis under low shear conditions that is similar to those present in the vena cava , large veins, and the arterial system.(110)This process is analyzed by a software.

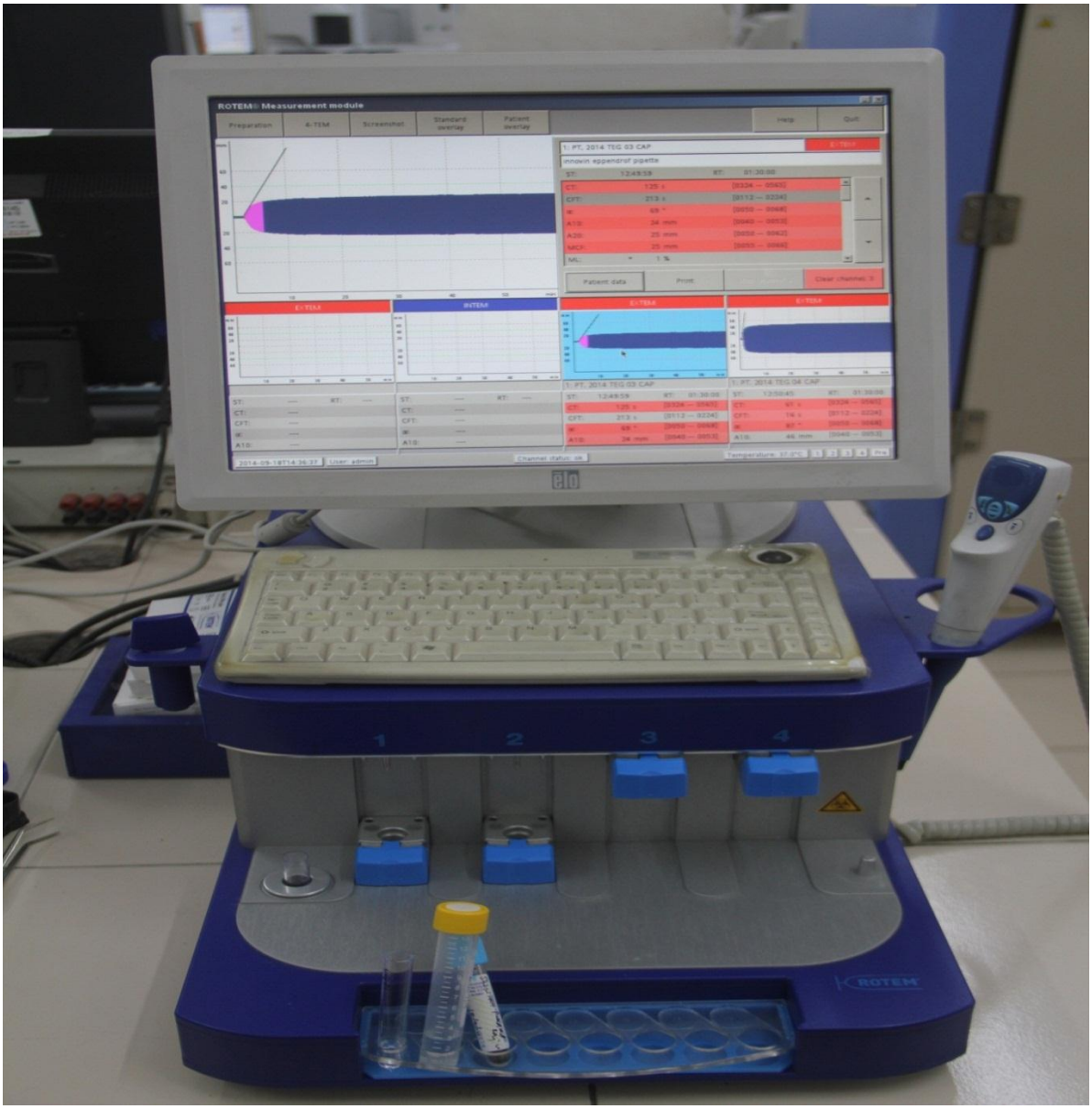

Figure 6- Thromboelastometry (ROTEM) machine

Technology

Rotational thromboelastometry evaluates the clot formation kinetics and strength by measuring the amount of a rotational force that is transmitted to an electromechanical transduction system by developing clot. . In this system, a cylindrical cup which is fixed and containing a 340 µl

whole blood sample and a pin suspended on a ball bearing mechanism initially oscillates through 4° 75′ every 6 sec through application of a constant force. As the strength of the clot increases the rotation of the pin is hindered it is detected optically using a image sensor system. ROTEM device is capable of analyzing 4 samples simultaneously. ROTEM provides automated pipetting. (111)

Parameters (Figure 6)

Clotting Time (CT)

It is the time from the beginning of the test by adding the clot activator until the time when amplitude of 2 mm is achieved.

Clot Formation Time (CFT)

It is the time between 2 mm amplitude and 20 mm amplitude of the clotting signal.

Alpha Angle (α)

It is defined as the angle between the middle axis and the tangent to the clotting curve through the 2 mm amplitude point. It describes the kinetics of clotting.

Maximum Clot Firmness (MCF)

It is the measure for the firmness of the clot and therefore the clot quality. It is the maximum amplitude that is reached before the clot is dissolved by fibrinolysis and the clot firmness falls again.

Maximum Lysis (ML)

It is the degree of fibrinolysis relative to maximum clot firmness achieved during the measurement.

ROTEM Assays

INTEM	Contact activation. Reagent - phospholipid and ellagic acid as activators Provides information similar to that of the APTT.
EXTEM	Tissue factor activation. Reagent contains tissue factor as an activator. Provides information similar to that of the PT.
APTEM	Aprotinin for inhibiting fibrinolysis Used in conjunction with EXTEM FOR assessing fibrinolysis.
HEPTEM	Contains lyophilized heparinase for neutralizing unfractionated heparin Used in conjunction with INTEM to assess heparin effect.
FIBTEM	Utilizes cytochalasin D, an actin polymerization inhibitor to block the platelet contribution to clot formation. Used in conjunction with EXTEM reagent for qualitative analysis of the fibrinogen contribution to clot strength independent of platelets.
NATEM	Native whole blood sample analyzed following only recalcification. Impractical for clinical use given long CFT time

The use of rotational thromboelastometry in transfusion support assessment allows targeted hemostatic approach. This strategy reduces blood usage as compared to a blinded approach to transfusion on the basis of laboratory tests with long turnaround times. The usual turnaround time for PT, aPTT, fibrinogen concentration and platelet count is generally long (nearly 45- 60 min) to determine the therapy in severely bleeding patients. The initial variables of ROTEM are available within 15–20 min.(112)

APPLICATION IN CLINICAL CONDITIONS

CARDIAC SURGERY

Hemorrhage following cardiac surgery is a common clinical crisis. There are various studies on the use of ROTEM for analysis of hemorrhage which appear to reduce the transfusion requirements as compared to standard coagulation profile. Studies have shown that rotational thromboelastometry can predict thrombocytopenia and hypofibrinogenemia in cardiac surgery using the clot amplitude after 5 minutes and the turnaround time of ROTEM was less than the conventional laboratory tests. (112) Studies in cardio thoracic patients have shown that ROTEM helps in detection of causes for postsurgical hemorrhage (113) Another study showed that thromboelastometry can be used in the study of the coagulation profile of cardiac surgery patients in post perfusion period. This gives information regarding hemostasis disorders and aids in the management.(114)

Thromboelastometry was also used to ascertain the effect of ex vivo addition with fibrinogen or platelet concentrate that caused shortened clotting time and improved clot strength in a dose-

dependent manner. This combination of fibrinogen and platelets improved the clotting time and strength. The addition of platelets promoted platelet aggregation in a dose-dependent manner while fibrinogen had no effect. The combination of fibrinogen and platelets improved platelet aggregation less than when the platelets were used alone. In cardiac surgery patients, the use of platelet and fibrinogen concentrates on blood samples had an additive effect on clot formation as compared to the individual components. These results had an impact on clinical transfusion protocols.(115)

TRAUMA

Trauma induced coagulopathy is caused by systemic anticoagulation and hyperfibrinolysis which occurs due to endothelial injury and protein C activation following hemorrhage from trauma sites, fluid replacement, hypothermia and tissue acidosis following hypo perfusion .(116) Some studies have shown that ROTEM analysis was better in comparison to standard coagulation tests in diagnosing trauma induced coagulopathy.(117) In some centers, ROTEM analysis is proved to be useful in diagnosing Trauma induced coagulopathy and was used in transfusion.(117) Hyperfibrinolysis associated with trauma can be diagnosed with the help of ROTEM.(118) The A10 variable correlated with platelet count and fibrinogen concentration and detection help in earlier goal-directed transfusion therapy and may allow modification of existing transfusion algorithms.(119)

OBSTETRICS

Postpartum hemorrhage (PPH) associated coagulopathy correlates poorly with standard laboratory coagulation tests and ROTEM based algorithms is used for the management of

specific coagulation deficiencies in these patients. This test is useful in hypofibrinogenemia and hyperfibrinolysis .The coagulopathy of PPH is can be due to dilutional, surgical, amniotic fluid embolus, and placental abruption causes and the ROTEM requires nearly 24 hours monitoring.(120)A study by Theusinger et al showed that maximum clot firmness in all channels were associated with fibrinogen and platelets levels, Clotting time significantly to aPTT and maximum clot firmness significantly to fibrinogen. Factor VIII also co related well with all ROTEM parameters except with Clotting time and Clot formation time. (121)

LIVER TRANSPLANTATION

In orthotopic liver transplantation, intraoperative bleeding is a major problem. The underlying liver pathology, the replacement of large blood losses, and the altered metabolism of coagulation factors during the prehepatic, anhepatic, and neohepatic phases of liver transplantation create a complicated hemostatic environment.(98) ROTEM assays can differentiate hypofibrinogenemia from thrombocytopenia based on the channels used.(122) A study by Blasi et al concluded that clot thickness at 10 minutes can be used to taken decisions regarding transfusions.(123)

Recent studies suggest ROTEM diagnose trauma induced coagulopathy more precisely and faster than standard coagulation tests. These assays can be used to take decisions regarding transfusion therapy as per the patient needs. This approach can therefore reduce the risk of over or under transfusion.(124) In comparison with standard coagulation tests, ROTEM parameters precisely detect trauma induced coagulopathy. The critical thresholds defined by ROTEM assays in bleeding patients were closely related with mortality and need for early RBC transfusion. Low maximum clot firmness is as an important deciding factor regarding red cell transfusion.(125)

The most important pathology in poly trauma patients are fibrinogen deficiency, poor fibrinogen polymerization and hyperfibrinolysis. A study by Tauber et al showed that when compared with standard coagulation profile, ROTEM parametersdetects trauma induced coagulopathy. The results can be obtained within a short time and reflects the in vivo situation in poly trauma patients. These patients predominantly showed hypofibrinoginemia and defective fibrin polymerization. In this study they found that a Maximum Clot Firmness of less than 7 mm, corresponds to a fibrinogen level of less than 150 mg/dl and those subjects had a higher mortality. Prothrombin time, fibrinogen concentrations and platelet count co- related with MCF. There was a high mortality rate for hyperfibrinolysis in this study, it and this can be reduced by early administration of tranexamic acid. (126)

OTHER CLINICAL CONDITIONS

Thromboelastometry can be also be used for diagnosis of pronounced hyperfibrinolysis. (127) Another study by Raza et al have shown that ROTEM is insensitive until fibrinolytic activity reaches a certain threshold but the addition of aprotinin, a stoichiometric inhibitor of plasmin in the APTEM assay have improved detection of hyperfibrinolysis. (128) Massive hemorrhage is a leading cause of mortality after cardiovascular surgery. The standard coagulation tests require a long turnaround time and cannot detect multiple hemostatic abnormalities. In a study by Sartorius et al in cardiothoracic surgical patients , clot firmness and maximum amplitude have been found to best correlate with platelet function and fibrinogen levels.(129) .When compared to standard coagulation tests, ROTEM can assess rate and quality of clot formation. This test is performed in whole blood and assess the collective effect of circulating platelets, RBC, leukocytes on clot formation, including platelet function (130)

A study by Mittermayr et al used Thromboelastometry heparin-protamine management. The variable used was CT. The ratio of CT in INTEM channel to CT-HEPTEM channel was used to differentiate the effects of heparin excess from those of protamine excess. When the ratio was more than 1 it is due to excess heparin while ratio equal to 1 was due to excess protamine.(131)

Graphical presentations in various clinical conditions

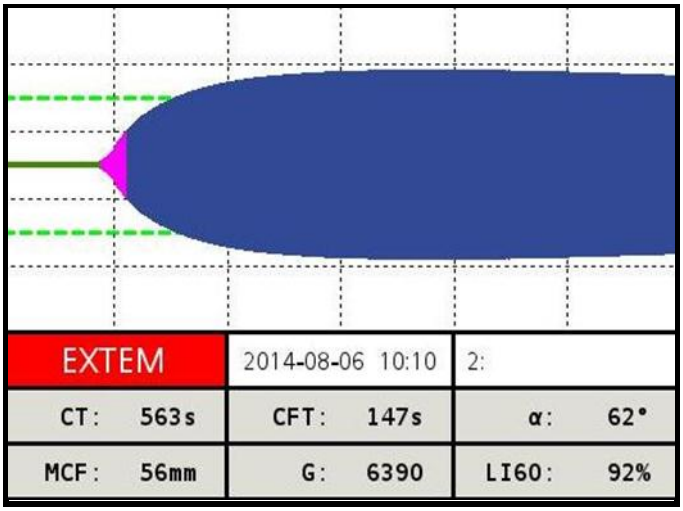

Figure 7 - A normal thromboelastogram

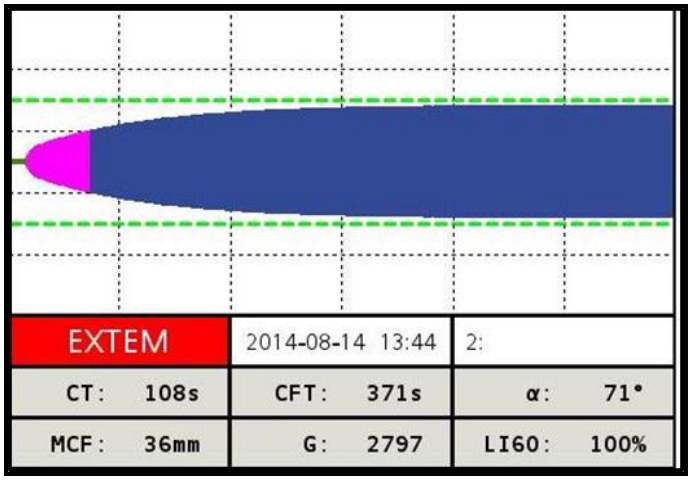

Figure 8 – Thromboelastogram with prolonged CFT and decreased MCF

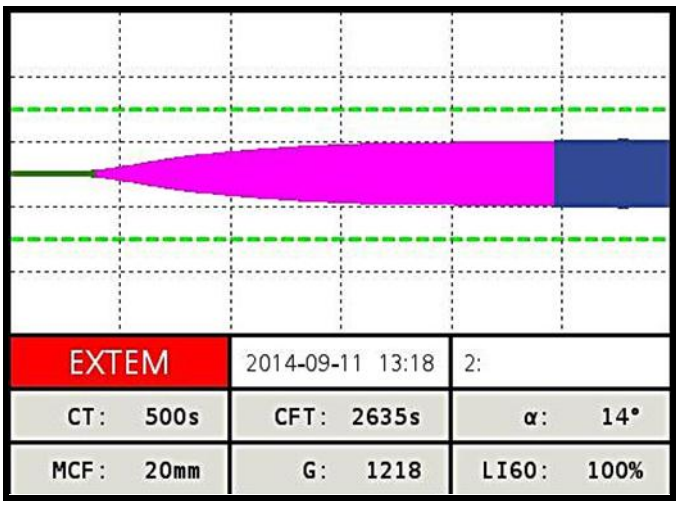

Figure 9 – Thromboelastogram with prolonged CT,CFT and decreased MCF and alpha angle

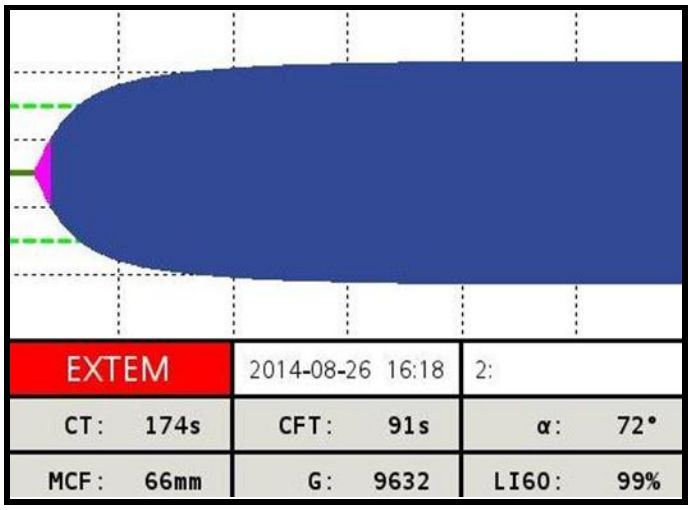

Figure 10 – Thromboelastogram with shortened CT and CFT

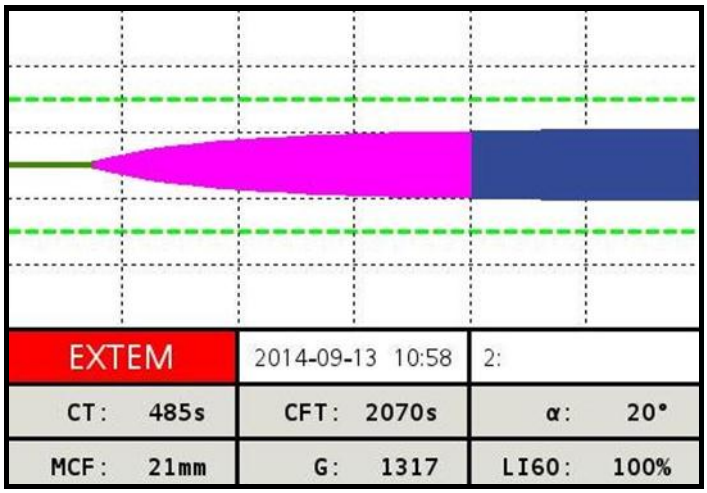

Figure 11-Thromboelastogram with prolonged CFT and decreased MCF and alpha angle

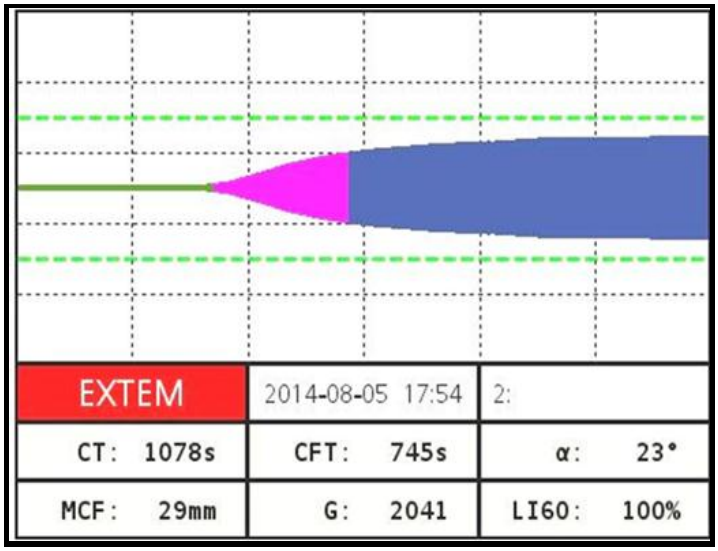

Figure 12 – Thromboelastogram with prolonged CT, CFT, decreased MCF and alpha angle as in severe coagulopathy

MATERIALS AND METHODS

This was a prospective study of patients who presented with acquired bleeding disorders in Christian Medical College, Vellore. The study was conducted on seventy newly admitted consecutive bleeding patients in the wards, the emergency and intensive care units of Christian Medical College Hospital for a period of two years from 01 January 2013. A detailed history, diagnosis, complete blood count profile, transfusion requirements, condition at discharge were collected with the help of a prepared pro forma.

STUDY POPULATION

TEST SAMPLES

Seventy patient samples of acquired bleeding disorder cases received in the Special Test Laboratory, Department of Transfusion Medicine and Immuno hematology between January 2013 – August, 2014 that have shown abnormal coagulation profile were considered as the test samples. The samples were collected on the basis of requests for blood products received in the blood bank from emergency, intensive care units and all surgical/ medical including super specialty departments. The complete bleeding history was taken from the treating physician/surgeon. Samples for the tests were obtained before transfusing the blood products.

CONTROL SAMPLES

In our hospital, the Department of Transfusion Medicine and Immuno hematology manages the Clinical Pathology Lab, Blood Bank, HLA lab and Special Test Lab for Coagulation work up. Patients who are referred from outside hospitals and out patients from other clinical departments who had presented with minor bleeding symptoms in the past that did not require any medical , surgical or transfusion interventions or no bleeding symptoms and had come for complete coagulation work up in Special test Lab were selected as the control group. On the basis of a complete coagulation work up, which included, complete blood count profile, standard coagulation profile, coagulation factor assays, Ricof levels and Platelet aggregometry studies, this category was diagnosed to have a normal coagulation profile. The first 68 consecutive samples during the study period were selected as the control samples.

Inclusion criteria:

Samples with abnormal thromboelastogram.

Exclusion criteria:

1. *Paediatric cases* – The normal reference range for prothrombin time, activated partial thromboplastin time, platelet count and serum fibrinogen levels are different from the adult ranges. There are no well defined pediatric reference ranges for rotem variables.

2. *Repeat sample collected within fifteen days* - Interventions like transfusion, medication will affect the coagulation parameters especially stable coagulation factors like factor XIII and fibrinogen. Cases on medication and those who had received transfusion were excluded from the control group.

Detailed history including type of bleeding with duration, diagnosis , treatment details like transfusion support with components, antifibrinolytics, vitamin K, factor concentrates were taken in detail.

Setting where data was collected

Special test lab, Department of Transfusion medicine and Immuno haematology, Christian Medical college, Vellore.

Sampling strategy employed

All consecutive samples with abnormal thromboelastometry within the given time period were included as test samples. Consecutively selected 68 samples with normal coagulation profile from patients with minor acquired bleeding disorders were considered as controls. Selection was dependent on the reference standard which is the coagulation profile. Samples with abnormal coagulation profile were considered as cases and normal samples as controls. Data was collected from participants prospectively (before performing the diagnostic tests)

Detailed history including history of risk factors if any was taken. It included the presenting symptoms with duration, significant medication history especially antiplatelet drugs , vitamin K antagonists were taken. Treatment details like transfusion of blood, platelets, fresh frozen plasma, cryoprecipitate, factor concentrates, use of antifibrinolytics like tranexamic acid were also taken in detail. Investigations like Complete Blood Counts, Prothrombin time, INR, Activated partial thromboplastin time, Serum Fibrinogen and platelet count were done by

automated methods using Sysmex machine and Coulter. All samples including cases and controls were subjected to thromboelastometric analysis using the ROTEM machine.

STANDARD OPERATING PROCEDURE

ROTEM

Purpose

To check the coagulation state of a blood sample.

Principle

The ROTEM technology is based on a fixed cylindrical cup and a permanently oscillating vertical axis. The axis is supported by a high precision ball bearing and oscillates to the left and to the right through an angle of 4.75º. The rotation of the axis is driven by a motor that is connected to the axis via an elastic spring. For the measurement, a disposable plastic pin with 6 mm diameter is placed firmly on the axis and the blood sample is filled into a disposable 8 mm diameter cup and is then uplifted onto the measurement channel. Hence, the plastic pin is immersed into the blood sample. The rotation is detected optically via a mirror plate at the upper end of the axis, a diode as light source and a light sensitive sensor (CCD Chip). If no clotting takes place, the movement is not obstructed. When a clot is formed and attaches itself between pin and cup surfaces, the movement is obstructed. The result is a balance between the spring tension and the tension of the clot. As the clot becomes firmer, the rotational amplitude of the axis is reduced.

Sample

Citrated whole blood (platelet rich plasma can also be used)

Materials:

Citrated whole blood, Disposable cups and pins, 100-1000µl, 5-40µl pipettes, 0.2M Calcium Chloride, Diluted thromboplastin (1/2000), Personnel Protective Equipment (PPE)

Sample preparation

Blood Sample: Blood is preferably drawn via a 21G needle directly into vacuette Greiner tube containing 3.2% sodium citrate with minimal stasis. The temperature of the sample may influence the measurement results. Measure the blood sample directly after sampling. If this is not possible, preheat the blood sample for 5-10 min before measurement in the sample preheating station of the ROTEM delta.

Reagent preparation

Preparation of Tissue factor:

Dilute 10µl of recombiplastin into 490µl of Imidazole buffer in tube 1.

Add 50 µl of the contents in tube 1 to 950 µl of Imidazole buffer in tube 2 and mix well.

Add 500µl of the contents in tube 2 to 500 µl of imidazole buffer in tube 3 and mix well.

This diluted recombiplastin is used for the processing of a sample on ROTEM.

Procedure

Switch on the ROTEM system. Activate the ROTEM system with the main switch on the back of the device. Push the blue on/off button on the right hand side of the instrument. Log in to the system. Touch the screen if the screen saver is active. Select user. Enter password Measurement module screen will open.

Measuring Cell Preparation

Take the cup with the pin in it from the storage box. Push the pin in the cup onto the axis chosen for measurement. The status line under the channel is grey by default. In case the axis has been moved heavily when attaching the pin, the channel becomes inactive and the status line turns blue during the time of initialization. Place the cup with its opening facing upwards into the appropriate preheated cup holder. Leave the cup holders always in the temperature controlled area. Push and fix the cup in the cup holder using the MC Rod. The cup must fit tightly. Enter Patient Data: Touch one of the four channels. In the upper part of the screen entry fields for patient data {Patient ID, Patient name (first name, last name, and comment} are displayed. Touch the respective entry field. Enter patient data.

Procedure

Add 20µl of 0.2M CaCl2 pre warmed cup. Add 30µl of diluted recombiplastin to a separate plastic vial. Mix 500µl of well mixed whole blood the tissue factor. Add 320µl of the mixture to the CaCl2 and mix well. Place the cup holder onto the measuring position using the guiding rods. The cup holder is kept in measuring position by magnets. Press the “Start manual” icon the screen.

Interpretation

During measurement, the large Thromboelastogram is shown at the left upper side of the screen. In the upper right part of the screen, the current measurement results of the test parameters are shown.

ROTEM Parameters

Clotting Time (CT), Clot Formation Time (CFT), Alpha Angle (α) Maximum Clot Firmness (MCF) and Maximum Lysis (ML)

Clotted sample can interfere with the results.

PROTHROMBIN TIME (PT)

Purpose

To look for the overall efficiency of extrinsic pathway of coagulation and monitoring of oral anticoagulant therapy.

Platform - Sysmex CS 2000i

Principle

Clot based assay. This test reflects the overall efficiency of extrinsic system. It is sensitive to changes in factor V, VII and X, and less so to factor II. It is also unsuitable for detecting minor changes in fibrinogen level but may be abnormal if the fibrinogen level is very low or if there is an inhibitor present. The reagent influences the sensitivity of the test and technique used and is important to establish a reference range locally. The reagent, contains tissue factor and phospholipids.

Limits of detection - 5 to 180 Sec

Primary sample-

Citrated Plasma sample.

Type of container

Light blue top vacutainer and 1ml mini collect Greiner Vacutte for pediatric purposes.

Reagents and Materials

Dade Innovin: product is reconstituted as per product insert/instruction sheet. This reagent already contains CaCl2. Patient plasma, control plasmas as required.

Procedure

From Main Menu - Press 'Reagent' icon. The reagent carousal and diluent table is displayed. On the screen, select one reagent position within a rack. This will be highlighted in blue, then press 'Change/Add' button. Wait while the carousal is rotating (the led will turn red). Once the carousal comes to a stop, the LED will turn green. Now unlock cover and remove the rack. Place the PT reagent (Dade Innovin) bottle into the rack with barcodes facing outwards. Use the appropriate adapters to keep smaller reagent containers.

In case of using cups adapters, they are placed so that the flat edge is facing outwards. Place the reagent rack back into the reagent table, replace the lid and lock it. A message will appear prompting reagent barcode reading. Press 'OK'. The reagent carousal will start running and will read the barcodes within the rack selected. Place the samples in the rack with the barcodes visible facing the front of the rack.

Place the rack on the right side of the sample tray ensuring that right edge of the rack is placed under guard rail of the sampler. Press 'Start' icon on the main menu. Select the tests to be performed from the grey test selection box. The selected tests will be highlighted with a blue dot. Press 'OK' button to return to the Order screen. Press 'Save' to confirm the order of the tests. Press 'Start' icon to commence the analysis. Input the appropriate rack number when the sampler dialogue box appears.

To view sample results, press the ' Joblist' icon on the main menu or the taskbar. The Joblist screen will be displayed. The status of the test is displayed in the far left hand column. The Test results can be viewed by scrolling across the screen using 'backward' and 'forwards' arrows at the bottom of the screen. Samples that have not been analyzed will be shown as a 'tick' along with a blue pending box. The result for any sample that requires reviewing will have an asterisk next to it. The status bar will display a yellow box stating 'Review'. The 'Review' or 'On hold' status will provide alerts regarding the flags attached to that particular result. The tests may be put on hold if a new lot no. of reagent has been used but not calibrated. The status bar on the left will be clear for analysis that has been completed with no errors. The numerical values will be shown under the appropriate test column. Any result that has a red Error box attached will display ***.* instead of numeric result. They could be due to events such as rinse solution running out, reagent depletion, probe crash etc.

To review and look up errors and flags on sample result: From the Joblist main screen, select the sample no. that needs to be reviewed, it will be highlighted blue. Double click the highlighted line or click on the browser. The browser screen is displayed. The results in the browser screen are sorted into different user definable tabs. To view at result for a particular test, select the appropriate tab on the screen The results will be displayed in the order that the graphs have been

set to appear. If the test you require is not present then scroll using 'up/down' buttons. Double click on the Coagulation curve using the mouse or the touch screen, It will display the detailed sample screen. Click the red 'Detail' box to display the error message, For HIL errors. The system will flag Hemolysis. lcteric and Lipemia are present. If lipemia occurs, the system may perform wavelength switch to minimize interference, this occurs without intervention. For both hemolysis and icteric samples, sample will need to be visually checked before result is validated. To validate the result, close the error dialogue box. Then close the detailed sample joblist screen. Press 'Validate' button.

QC protocol

A normal control (pooled normal plasma [PNP]) and an abnormal control plasma (Coag-path from Stago) is included with every batch of patient plasma tested, or every few hours if testing a large number of plasmas throughout the day. Normal control and abnormal control plasma are run for 10 days and the mean and +/-2SD is calculated . The control values should fall within +/-2SD.

Possible interference

Improper centrifugation, plasma from badly haemolysed blood, highly lipemic sample and blood not tested within four hours of collection.

Result interpretation

The results are expressed automatically on the “worklist” page as seconds. The results are always interpreted with INR (International Normalized Ratio).

Normal Range. 10 – 12.5 Secs.

ISI

INR = Prothrombin Time (PT) of test plasma (sec)

Mean Normal Prothrombin Time (MNPT) (sec)

ISI = International Sensitivity Index.

INR= International normalized ratio.

Ideally, each laboratory should establish its own Mean Normal Prothrombin Time (MNPT) obtained by testing at least 20 normal plasmas in that laboratory's PT assay, and taking the mean PT. Selected instrument specific ISI values are usually provided for each batch of Innovin.

Always use the necessary PPE for all procedures done in the laboratory. Consider every biological sample as a potential bio hazard.

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

Platform used

Sysmex CS 2000i

Purpose

To rule out overall efficiency of intrinsic and common pathway of coagulation and monitoring of heparin therapy.

Principle

This is a clot based assay which measures the clotting time of the plasma after the activation of contact factors but without adding tissue thromboplastin and so indicates the overall efficiency of intrinsic pathway. For the activation of contact factors, the plasma is first pre- incubated for a set period of time with a contact activator such as Kaolin or elagic acid. During this phase of the test, factor XIIa is produced which cleaves factor XI to XIa but coagulation does not proceed beyond this in the absence of calcium. After recalcification, F XIa activates factor IX and coagulation cascade proceeds. Since these tests are performed on platelet poor plasma a standardized phospholipid is included in the reagent.

Limits of detection

8 to 180 Sec

Primary sample

Citrated Plasma sample. Light blue top vacutainer and 1ml mini collect Greiner Vacutte for pediatric purposes.

Reagents and Materials: SynthAsil (IL), CaCl2 provided in the APTT reagent kit, Patient plasma, control plasmas as required.

Procedure

Loading Reagents on-board the CS 2000i analyzer: From Main Menu - Press 'Reagent' icon. The reagent carousal and diluent table is displayed. On the screen, select one reagent position within a rack. This will be highlighted in blue, then press'Change/Add' button. Wait while the carousal is rotating (the led will turn red). Once the carousal comes to a stop, the LED will turn green.

Now unlock cover and remove the rack. Place reagent bottles, Synth Asil and CaCl2 into the rack with barcodes facing inside. Use the appropriate adapters to keep smaller reagent containers. In case of using cups adapters, they are placed so that the flat edge is facing outwards. Place the reagent rack back into the reagent table, replace the lid and lock it. A message will appear prompting reagent barcode reading. Press 'OK'. The reagent carousal will start running and will read the barcodes within the rack selected. Once the barcodes are read the reagent screen will appear. The reagents that have been previously read by the analyzer will be displayed in its relevant position number with the name of the reagent underneath. A red question mark is displayed if the reagent placed in an adapter cannot be identified as there is no barcode or non-readable barcode. If the reagent has been previously placed onboard, then highlight the red question mark and press' Edit Reagent Info'. Place the cursor inside the reagent box and a drop down menu appears. Now select the appropriate reagent from the list. Click on the lot number box and select the lot number from selection list. Enter the type of container by selecting from the drop down menu. Press 'OK' to save. Place the samples in the rack with the barcodes visible facing the front of the rack.2. Place the rack on the right side of the sample tray ensuring that right edge of the rack is placed under guard rail of the sampler. lf the rack is in the correct position, the barcodes on the samples are facing towards the analyzer. The rack position numbers are from l on left to I0 on the right. Press

'Start' icon on the main menu. If samples are to be entered manually. From the main menu, select 'Order' icon. Press 'Order Entry' button on the following screen. For the manual entry of sample number, use the onscreen keypad or IPU key pad and enter the value in the blue 'sample No.' box. Use the 'Edit Sample info' box to amend the faulty entry. Select the tests to be performed from the grey test selection box. The selected tests will be highlighted with a blue dot.

Use the 'up' /' down' keys to move to subsequent sample positions and make the respective entries. Press 'OK' button to return to the Order screen. Press 'Save' to confirm the order of the tests. Press 'Start' icon to commence the analysis. Input the appropriate rack number when the sampler dialogue box appears. The Test results can be viewed by scrolling across the screen using 'backward' and 'forwards' arrows at the bottom of the screen. Samples that have not been analyzed will be shown as a 'tick' along with a blue pending box. The result for any sample that requires reviewing will have an asterisk next to it. The status bar will display a yellow box stating 'Review'. The 'Review' or 'On hold' status will provide alerts regarding the flags attached to that particular result. The tests may be put on hold if a new lot no. of reagent has been used but not calibrated. The status bar on the left will be clear for analysis that has been completed with no errors. The numerical values will be shown under the appropriate test column. Any result that has a red Error box attached will display ***.* instead of numeric result. They could be due to events such as rinse solution running out, reagent depletion, probe crash etc. the sample no. that needs to be reviewed, it will be highlighted blue. Double click the highlighted line or click on the browser. The browser screen is displayed. The results in the browser screen are sorted into different user definable tabs e.g., _ Routine. factors. Thrombo etc .,To view at result for a particular test, select the appropriate tab on the screen. The results will be displayed in the order that the graphs have been set to appear. If the test you require is not present then scroll using 'up/down' buttons. Double click on the Coagulation curve using the mouse or the touch screen, It will display the detailed sample screen. Click the red 'Detail' box to display the error message, For HIL errors. The system will flag Hemolysis. lcteric and Lipemia are present. If lipemia occurs, the system may perform wavelength switch to minimize interference, this occurs without intervention. For both hemolysis and icteric samples, sample will need to be visually checked

before result is validated. To validate the result, close the error dialogue box. Then close the detailed sample joblist screen. Press 'Validate' button.

QC protocol

A normal control (e.g. pooled normal plasma [PNP]) and an abnormal control plasma (e.g. Coag-path from Stago) should also be included with every batch of patient plasma tested, or every few hours if testing a large number of plasmas throughout the day. Run normal control and abnormal control plasma for 10 days and calculate the mean and +/-2SD. The control values should fall within +/-2SD.

Possible interference

Improper centrifugation of patient sample and badly hemolysed blood may give an artifactual coagulation results that do not accurately represent the coagulation status of the patient under investigation. Hemolysed blood may suggest a traumatic blood collection and you may need to request a repeat sample collection. Blood not tested within four hours of collection.

Result interpretation: Report APTT results in seconds. As a rough guide the aPTT of normal plasma should be 25 to 35 sec. In our lab any value more than the assigned reference range is considered as abnormal and needs further evaluation. Always use the necessary PPE for all procedures done in the laboratory. All samples must be processed within four hours of collection.

Fibrinogen

Platform

CS 2000i

Purpose

To estimate the amount of fibrinogen in citrated plasma sample.

Principle

Clot based assay.

Dilutions of standard normal plasma with known fibrinogen content are prepared in Imidazole buffer. The clotting time is measured after the addition of thrombin. A graph of clotting times against the fibrinogen concentration is constructed. The clotting time is proportional to the concentration of fibrinogen and the 1/10 dilution is taken to represent the value in the standard preparation. The test plasma is diluted 1/10 and the result read from the standard line.

Limits of detection

30-1400 mg/dL.

Primary sample

Citrated Plasma sample. Light blue top vacutainer and 1ml mini collect Greiner Vacutte for pediatric purposes.

Reagents and Materials

Standard or reference plasma with known fibrinogen concentration. Thrombin > 30 u/ml (concentration may vary according to source) Imidazole buffer (glyoxalin) pH 7.35

Procedure

From Main Menu - Press 'Reagent' icon. The reagent carousal and diluents table is displayed. On the screen, select one reagent position within a rack. This will be highlighted in blue, then press Change/Add' button. Wait while the carousal is rotating (the led will turn red). Once the carousal comes to a stop, the LED will turn green. Now unlock cover and remove the rack. Place the

reconstituted Fibrinogen reagent in Reagent table A. Imidazole Buffer in a 5 ml container is to be placed in STAT diluent table. Use the appropriate adapters to keep smaller reagent containers. In case of using cups adapters, they are placed so that the flat edge is facing outwards. Place the reagent rack back into the reagent table, replace the lid and lock it. A message will appear prompting reagent barcode reading. Press 'OK'. The reagent carousal will start running and will read the barcodes within the rack selected.

Calibration

The required reagents and calibrator are loaded on to the CS-2000i analyzer. The calibrators will need to be poured into 4ml cups and placed in the reagent table with suitable adapter.Once the barcodes of the reagents have been read, the system is ready to run the calibration for the assay and generate calibration curve. Click 'Order' icon from the main menu. In the Order screen, select the 'Switch Order' button. Select the 'Holder Calib. Curve Order' box. Click on the required 'Gray button' on the bottom of the screen to select the required assay for the calibration curve. Once selected, the assay box will have a blue dot in it. A small screen appears, click into the top box labeled 'Reagent lot'. Another box titled select reagent lot will appear. Select the required lot from drop down list and press 'OK' Select the appropriate lot no. for the current calibration. CS analyzer can hold up to 10 separate calibration curve per assay at a time. If no lot numbers are available in the drop down box it means that no reagents are registered on the reagent table for this particular assay. Click in the 'Lot' box. The screen will show the calibrator lots that are available and select the required lot number. Then Click in the

'Assay Sheet Value' box and enter the specific calibrator value. Select 'OK'. The Order screen will now show a check (\/) mark under the assays that has been selected for calibration. Select 'Close' button to go back to the main menu screen. Press 'Start' icon. Select the ' Joblist' icon to

view the status of the calibration curve. Select the 'Calib Curve' icon. The screen will display the respective calibration curve. Review and validate the curve. Place the samples in the rack with the barcodes visible facing the front of the rack. Place the rack on the right side of the sample tray ensuring that right edge of the rack is placed under guard rail of the sampler. If the rack is in the correct position, the barcodes on the samples are facing towards the analyzer. The rack position numbers are from l on left to 10 on the right. Press 'Start' icon on the main menu. If samples are to be entered manually. From the main menu, select 'Order' icon. Press 'Order Entry' button on the following screen. For the manual entry of sample number, use the onscreen keypad or IPU key pad and enter the value in the blue 'sample No.' box. Use the 'Edit Sample info' box to amend the faulty entry. Select the tests to be performed from the grey test selection box. The selected tests will be highlighted with a blue dot. Use the 'up' /' down' keys to move to subsequent sample positions and make the respective entries. Press 'OK' button to return to the Order screen. Press 'Save' to confirm the order of the tests. Press 'Start' icon to commence the analysis.. Input the appropriate rack number when the sampler dialogue box appears. The Test results can be viewed by scrolling across the screen using 'backward' and 'forwards' arrows at the bottom of the screen. Samples that have not been analyzed will be shown as a 'tick' along with a blue pending box. The result for any sample that requires reviewing will have an asterisk next to it. The status bar will display a yellow box stating 'Review'. The 'Review' or 'On hold' status will provide alerts regarding the flags attached to that particular result. The tests may be put on hold if a new lot no. of reagent has been used but not calibrated. The status bar on the left will be clear for analysis that has been completed with no errors. The numerical values will be shown under the appropriate test column. Any result that has a red Error box attached will display ***.* instead of numeric result. They could be due to events such as rinse solution running out, reagent

depletion, probe crash etc. For both hemolysis and icteric samples, sample will need to be visually checked before result is validated. To validate the result, close the error dialogue box. Then close the detailed sample joblist screen. Press 'Validate' button.

QC protocol

A normal control (e.g. pooled normal plasma [PNP]) and an abnormal control plasma (e.g. Coag-path from Stago) should also be included with every batch of patient plasma tested, or every few hours if testing a large number of plasmas throughout the day. Run normal control and abnormal control plasma for 10 days and calculate the mean and +/-2SD. The control values should fall within +/-2SD.

Possible interference

Improper centrifugation of patient sample and badly haemolysed blood may give an artifactual coagulation results that do not accurately represent the coagulation status of the patient under investigation. Haemolysed blood may suggest a traumatic blood collection and you may need to request a repeat sample collection. Blood not tested within four hours of collection.

Result interpretation

The normal range should be established locally but is usually close to 150-450mg/ml. For most

Clauss techniques, the relationship between clotting time and fibrinogen concentration is linear over a limited range of clotting times, typically 10-25 seconds. For normal test plasma a 1/10 dilution can be used (automatically). For lower concentrations, for example 75-150mg/ml the plasma should be diluted 1 in 5. For levels < 75mg/ml, the test plasma should be diluted 1/2. For higher levels (> 400mg/ml), the test plasma should be diluted 1/20. These calculations correct for the difference in dilution of the test and standard plasma samples.

Always use the necessary PPE for all procedures done in the laboratory. All samples must be processed within four hours of collection.

Platelet Count

Principle

Perform total Platelet count on a Neubauer chamber.

Primary sample

K2EDTA blood.

Sample and equipment requirement:

Platelet diluting fluid, a. 1% formalin, 3% sodium citrate, 1-2 drops brilliant cresyl blue ,Sahali pipette ,Whole blood, Microscope (45X),Neubauer chamber

Procedure

Mix the blood specimen carefully. Using sahali pipette dilute the blood 1: 100 with platelet diluting fluid. Mix well, after 5 mins charge it on a counting chamber. Place the filled chamber under petridish with a moist filter paper. Let it stand for 10 to 15 minutes. Place the counting chamber carefully on the stage of microscope. Focus the area under 45 X. Keep the condenser down, reduce the light by adjust the diaphragm. Platelet will appear like highly refractile particle. Count the platelets on the central RBC square or any of the WBC squares.

QC protocol:

Slide count should be done (1 platelet is equal to 15000 in oil immersion field). Blank count should be done to check the platelet diluting fluid. Platelet count can be done in cell counter to check whether they correlate. Duplicate checking.

Specimen diluted

IV line, aged and lysed specimen interfere with the results.

Result interpretation

Platelets = $\frac{\text{Number of cell counted x dilution factor x depth factor}}{\text{Area counted}}$

= $\frac{\text{Number of cell counted x 100 x 10}}{1}$

Table -2 . Statistical analysis plan for calculating sensitivity and specificity of Rotational Thromboelastometry compared to Standard coagulation profile

2X2 TABLE FOR ANALYSIS		STANDARD COAGULATION PROFILE (GOLD STANDARD)		TOTAL
		POSITIVE	NEGATIVE	
ROTEM (TEST)	POSITIVE	A	B	a + b
	NEGATIVE	C	D	c + d
Total		a+c	b+d	a+b+c+d

Sensitivity = a/a+c

Specificity=d/b+d

A fixed number of cases and controls are sampled between the patients with acquired bleeding disordrs and the normal subjects. When controls are easier to obtain, more controls can be considered than cases (Janes et al, 2005).

RESULTS

We studied a total of 138 samples which included 70 cases and 68 controls from January 2013-August 2014.

Results:

Total number of patients studied -138

Cases (altered standard coagulation profile) -70

Controls (normal coagulation profile) – 68

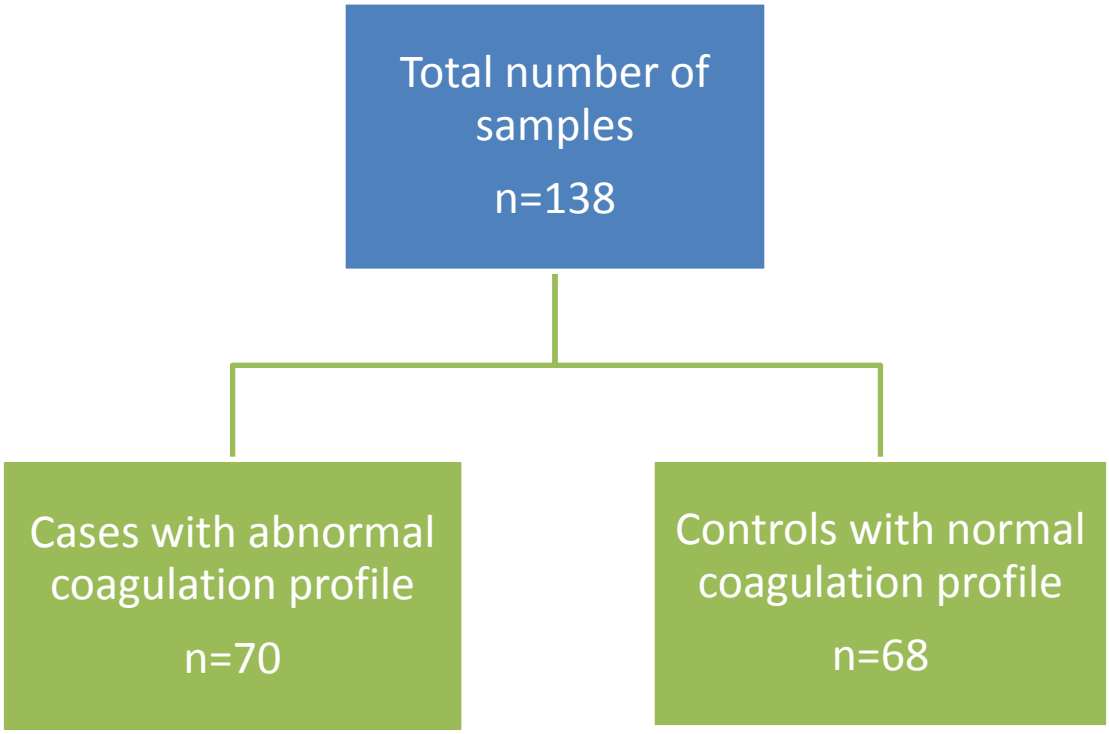

Figure 13- Flow chart of cases and controls category

In the seventy cases, certain cases had one, two or three altered tests(Prothrombin time, activated Partial thromboplastin Time, Platelet count and Serum Fibrinogen levels) , but none had all the four coagulation parameters altered.

Total number of patients studied - 138
Number of cases who had an altered complete standard coagulation profile – 70
Number of control who had a normal complete standard coagulation profile- 68
Number of cases who had prolonged Prothrombin time – 64
Number of cases who had prolonged Activated Partial Thromboplastin Time – 54
Number of cases who had low platelet count (less than 1,00,000/cumm) -44
Number of cases who had low Fibrinogen levels(less than 150 mg/dl) -35

Table 3 - Distribution of altered coagulation profile parameters

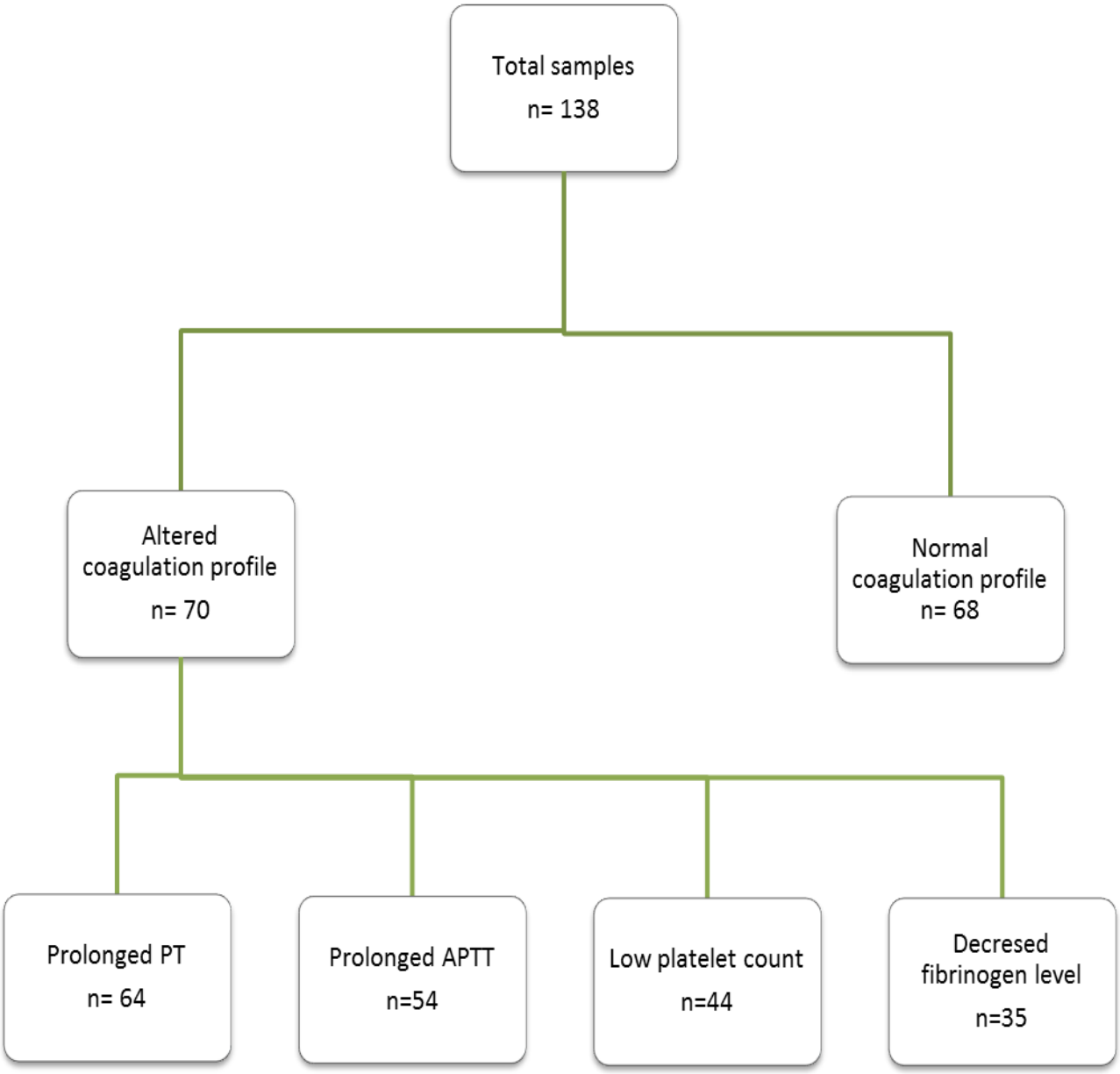

Figure 14 . Flow chart of cases showing altered coagulation profile

The baseline characteristics of cases and controls are given in table no 4.. The mean age of cases was 41.3±16.2 years and that of controls was 33.3 ±13.7 years. Females accounted for 34.3% of the cases and 54.4% of the controls (Figure 15). In both cases and control group, a majority of cases were seen in the age group 21 -30 years. (Figure 16)

Characteristic		**Total n(%)**	**Cases n(%)mean±sd**	**Controls n(%)mean±sd**
Total Number		138	70	68
Age (years)		37.3 ± 15.5	41.3± 16.2	33.3±13.7
Gender	Male	77(55.8%)	46 (65.7%)	31(45.6%)
	Female	61 (44.2%)	24 (34.3%)	37 (54.4%)

Table 4 - Baseline Characteristics

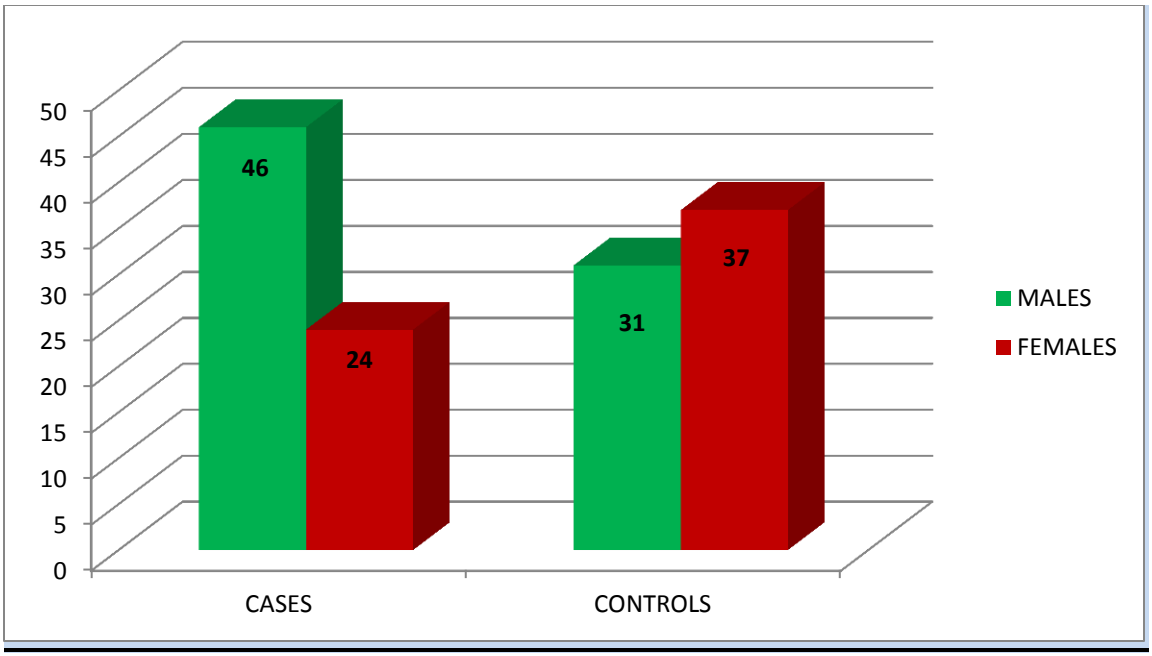

Figure 15- Sex distribution among cases and controls

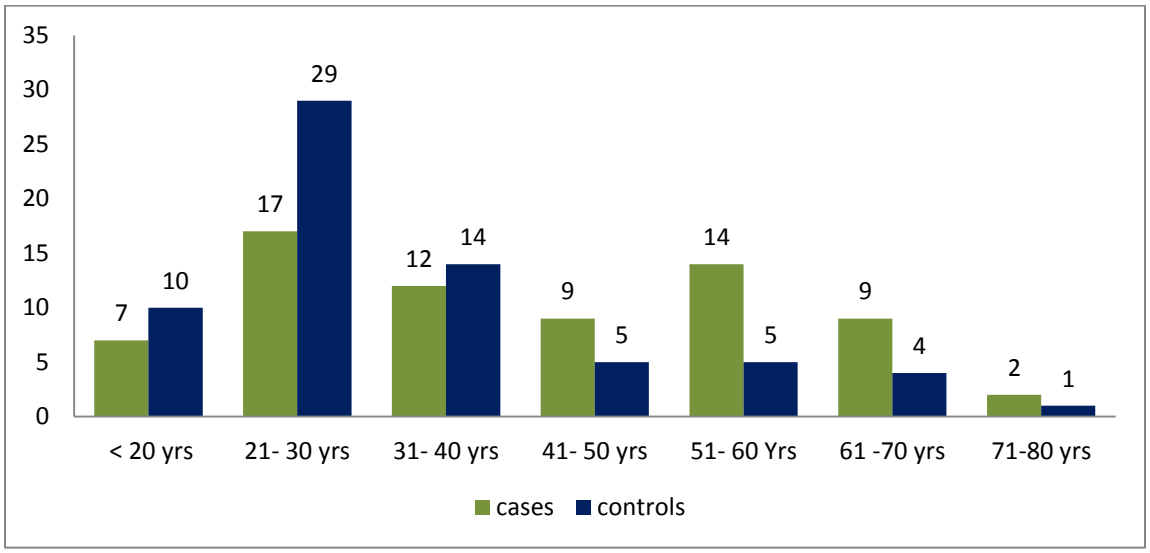

Figure 16- Distribution of age in cases and controls

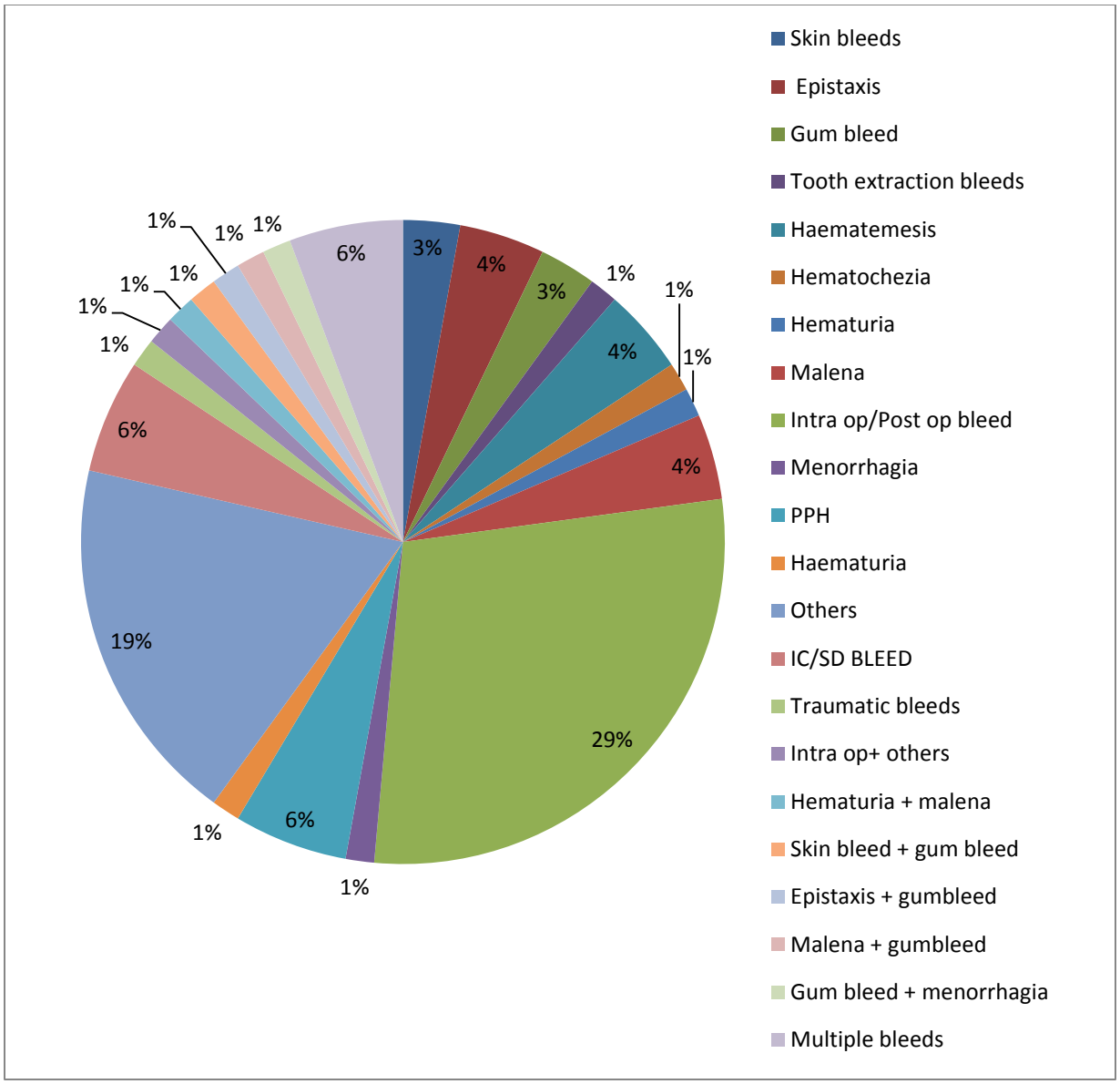

Figure 17- Distribution of bleeding manifestations among cases.

The bleeding manifestations ranged from minor mucosal bleeds like epistaxis to life threatening operative hemorrhages requiring transfusion support. A major proportion of our cases had severe intraoperative and postoperative bleeds leading to coagulopathy. Twelve percent of the total cases had more than one bleeding manifestation.

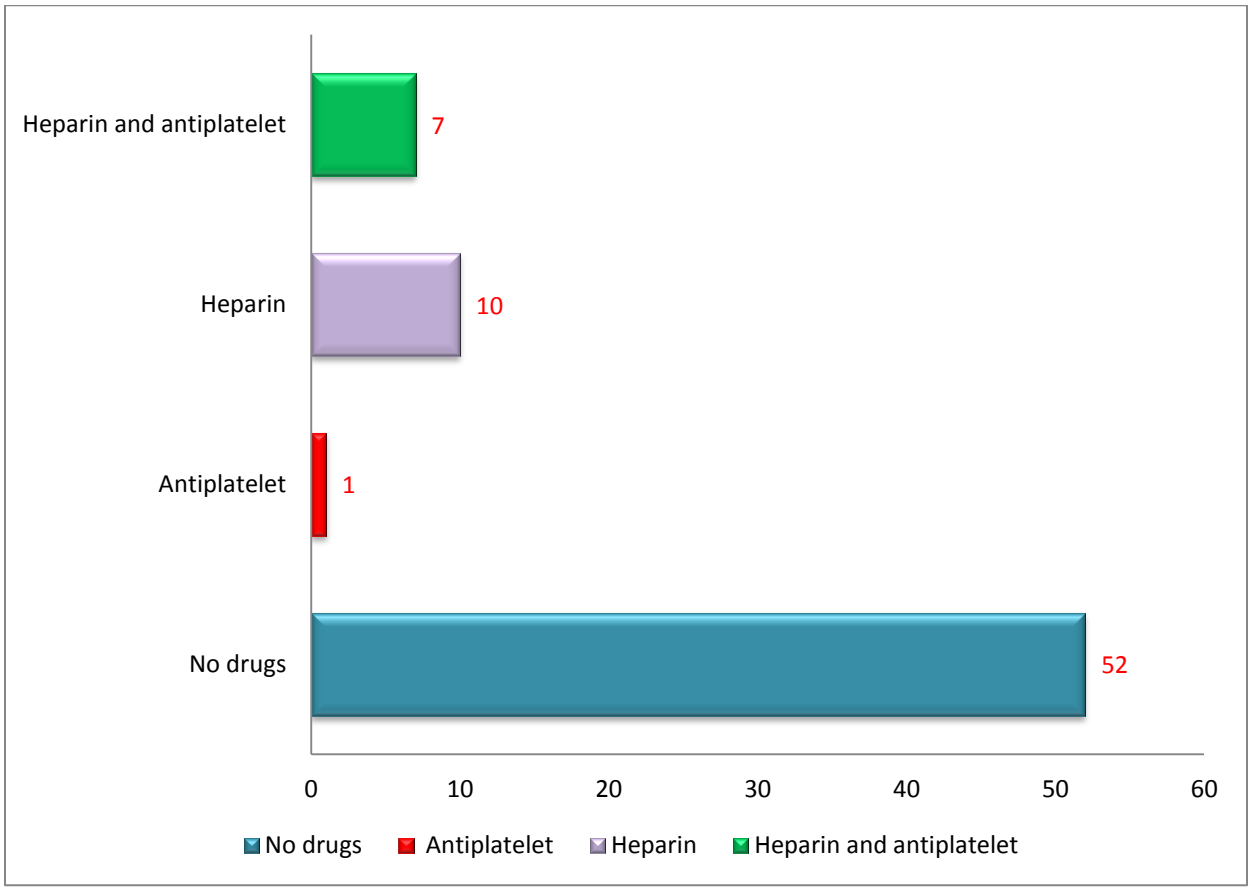

Figure18 - Graphical representation of medication usage in cases.

Among the cases, nearly 52% of the patients were not on any medications that would have caused bleeding. Ten percent of the patients were on Heparin for their primary disease, 1% on antiplatelet drugs like Aspirin and Clopidogrel while 7% of patients were on both drugs. In the control group all 100% were not on any medication which we had kept as an exclusion criteria while selecting the donors.

DIAGNOSIS	CASES	PERCENT
ORTHOPTIC LIVER TRANSPLANATION	5	7
VIT K DEPENDANT FACTOR DEFICIENCY	1	1
TRAUMA INDUCED COAGULOPATHY	22	31
ACQUIRED HAEMOPHILIA A	2	3
MALIGNANCY ASSOCIATED COAGULOPATHY	12	17
LUPUS ANTICOAGULANT	2	3
SUB ACUTE DIC	1	1
OTHER FACTOR DEFICENCY	2	3
SEPSIS ASSOCIATED COAGULOPATHY	6	9
COAGULOPATHY ASSOCIATED WITH LIVER DISEASE	7	10
COAGULOPATHY ASSOCIATED WITH OBSTETRIC CASES	8	12
COAGULOPATHY ASSOCIATED WITH SNAKE BITE	2	3

Table 5- Clinical diagnosis leading to bleeding in cases

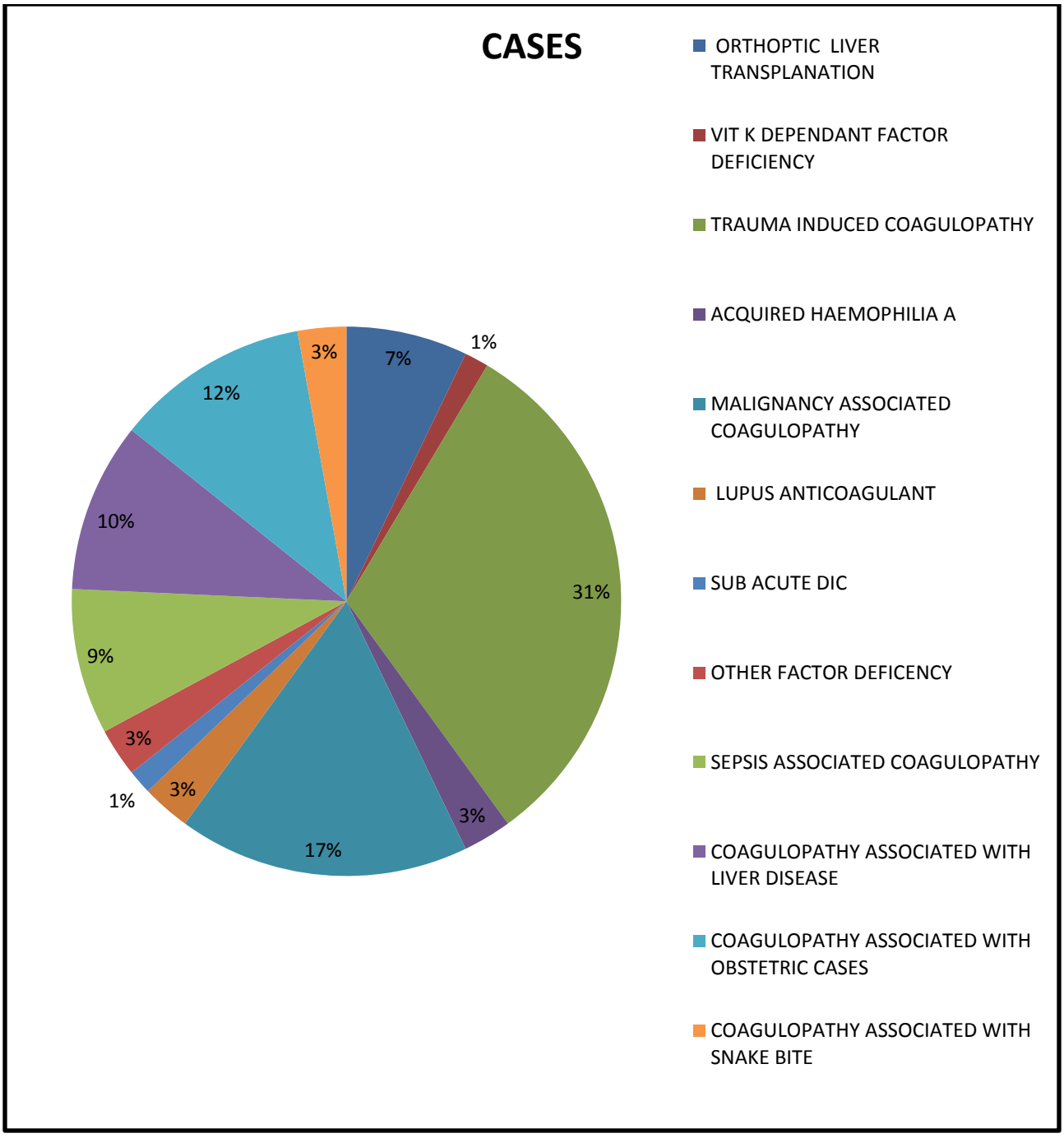

Figure 19- Distribution of diagnosis in cases

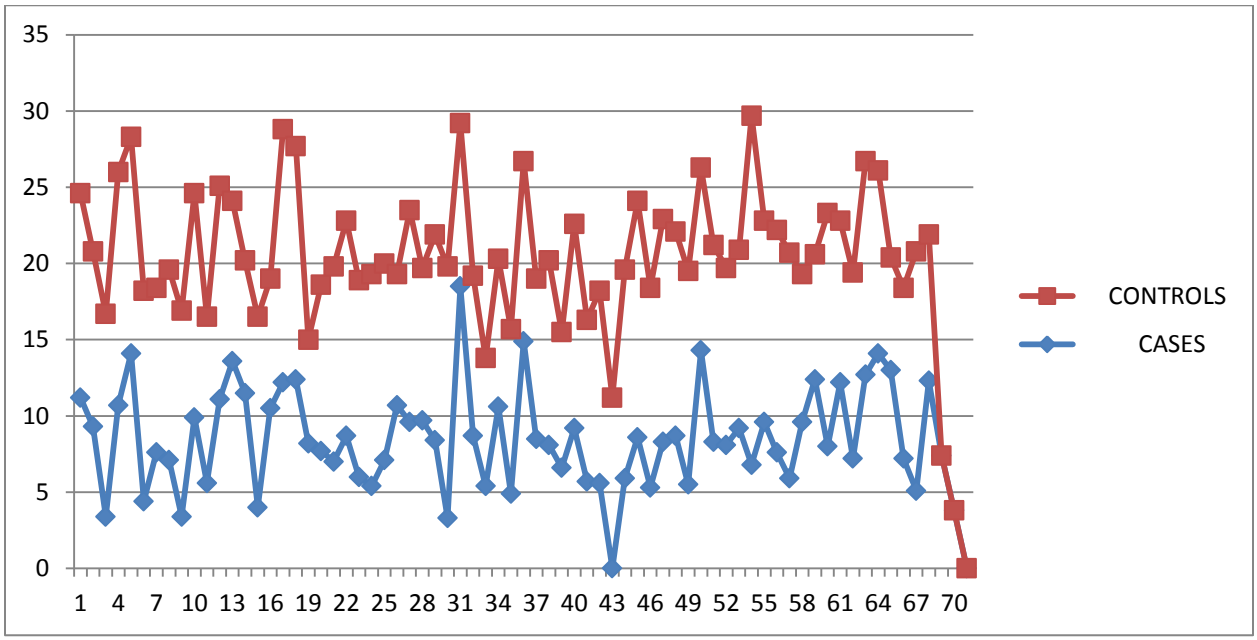

Figure 19- Distribution of Haemoglobin among cases and controls

Hb in mg/dl	Frequency	Percent
5	07	10
5- 10	42	60
10	21	30
Total	70	100.0

Table 6- Frequency distribution of Hb levels in cases

	Total n(%)	Cases n(%) or mean±sd	Controls n(%) or mean±sd
Total Number	138	70	68
Hb	10.5 ± 3.4	8.7± 3.1	12.4±2.6

Table 7- Range of Haemoglobin among cases and controls

All the controls had a normal haemoglobin levels while the cases it was decreased due to the bleeding manifestations. A ten percent of the cases had Hb less than 5 mg/dl.

RANGE (/cumm)	FREQUENCY	PERCENT
< 50,000	**24**	**34.3**
50,000-1,00,000	**20**	**28.6**
> 1,00,000	**26**	**37.1**

Table 8- Frequency distribution of platelet count in cases

All the controls had a normal platelet count while the cases it was decreased due to the bleeding manifestations. A ten percent of the cases had Hb less than 5 mg/dl.

Range	Frequency	Percent
10- 12.4	6	8.6
>12.4	64	91.4

Table 9- Frequency distribution of normal and altered Prothrombin time among cases

Out of the seventy cases, six cases had a normal Prothrombin time as compared to the rest sixty four which had a prolonged prothrombin time.

DIAGNOSIS	FREQUENCY
ACQUIRED HAEMOPHILA A	2
COAGULOPATHY ASSOCIATED WITH MALIGNANCY	2
LUPUS ANTICOAGULANT	1
COAGULOPATHY ASSOCIATED WITH OBSTETRIC CASES	1

Table 10 – Disorders which had a normal Prothrombin time

Fifty three out of the sixty four cases, the prothrombin time was corrected on addition of normal plasma. Eleven cases did not show any correction and the clinical condition associated with this scenario is shown in Table 11

DIAGNOSIS	NUMBER
Orthoptic liver transplanation	**2**
Trauma induced coagulopathy	**5**
Coagulopathy associated with liver disease	**2**
Sepsis associated coagulopathy	**1**
Coagulopathy associated with obstetric cases	**1**

Table 11- Cases with no correction Of Prothrimbin time

Six out of seventy cases had normal INR (range- 0.99- 1.12) corresponding with the PT while sixty four cases had a higher INR (range 1.16-10).The normal range of aPTT is 25.1 secs - 36.6 secs. Fifty four out of seventy patients had a prolonged aPTT (range 37.2 secs - more than 2min) while sixteen cases had a normal result (Range- 23.9-35.5secs).

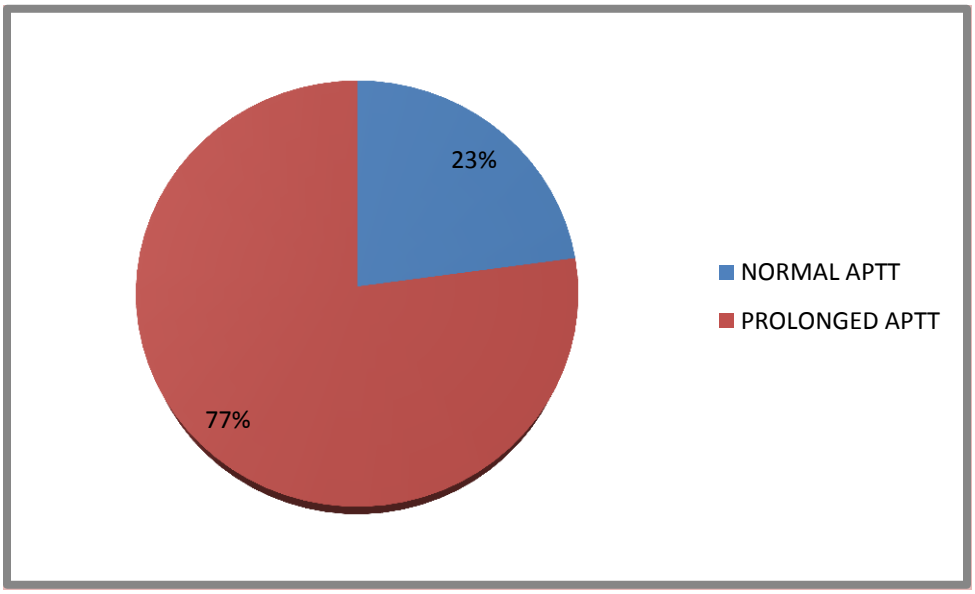

Figure 20- Pie chart showing the number of bleeders with normal and altered aPTT

Five out of fifty four cases, the aPTT was not corrected with normal plasma and the disorders which caused the prolongation are shown in table

DISORDERS	FREQUENCY
Trauma induced coagulopathy	1
Acquired haemophila A	2
Lupus anticoagulant	1
Sepsis associated coagulopathy	1

Table 12 – Disorders which had a prolonged aPTT and not corrected with normal plasma

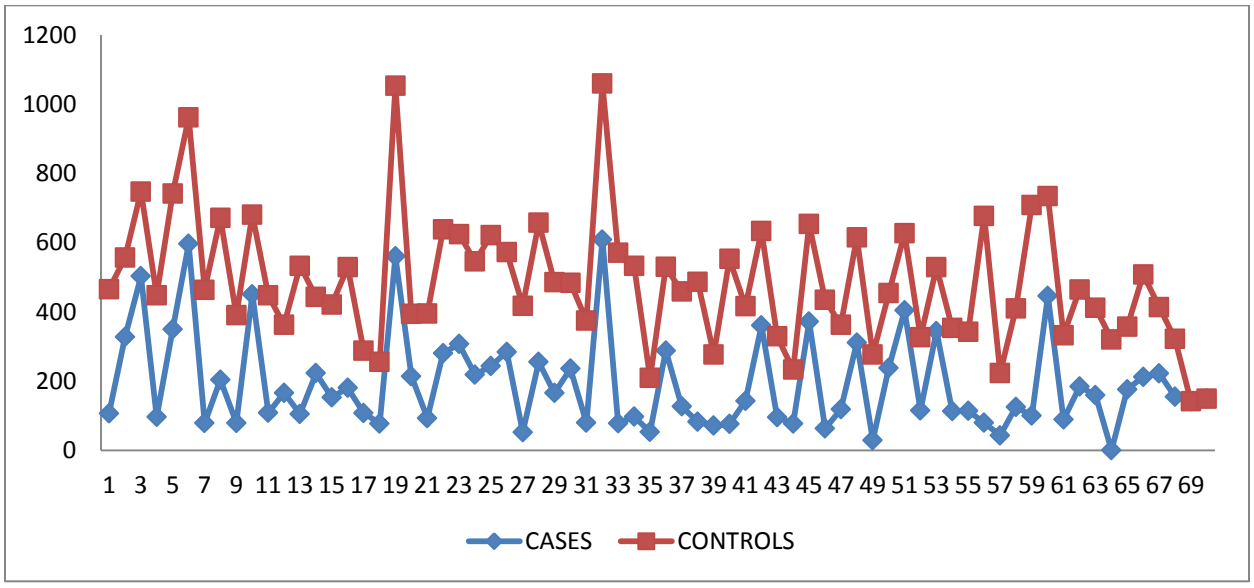

Figure 21- Distribution of serum Fibrinogen among cases and controls

All the controls had a normal fibrinogen level, 4.3% of cases had a fibrinogen level of less than 50 mg/dL. One case out of this group had undetectable fibrinogen. Nineteen patients (27.1 %) the levels of fibrinogen were between 51- 100 mg/dl and thirteen patients between 101-150 mg /dL. The remaining fifty percent had a normal fibrinogen level in spite of the bleeding episode.

FIBRINOGEN LEVELS(mg/dL)	NUMBER	PERCENT
LESS THAN 50	**3**	**4.3**
51-100	**19**	**27.1**
101-150	**13**	**18.6**
>150	**35**	**50**

Table 13- Fibrinogen levels in cases

CT VALUES	FREQUENCY	PERCENT
NORMAL	**54**	**77.14**
PROLONGED	**16**	**22.86**

Table 14- CT values among cases

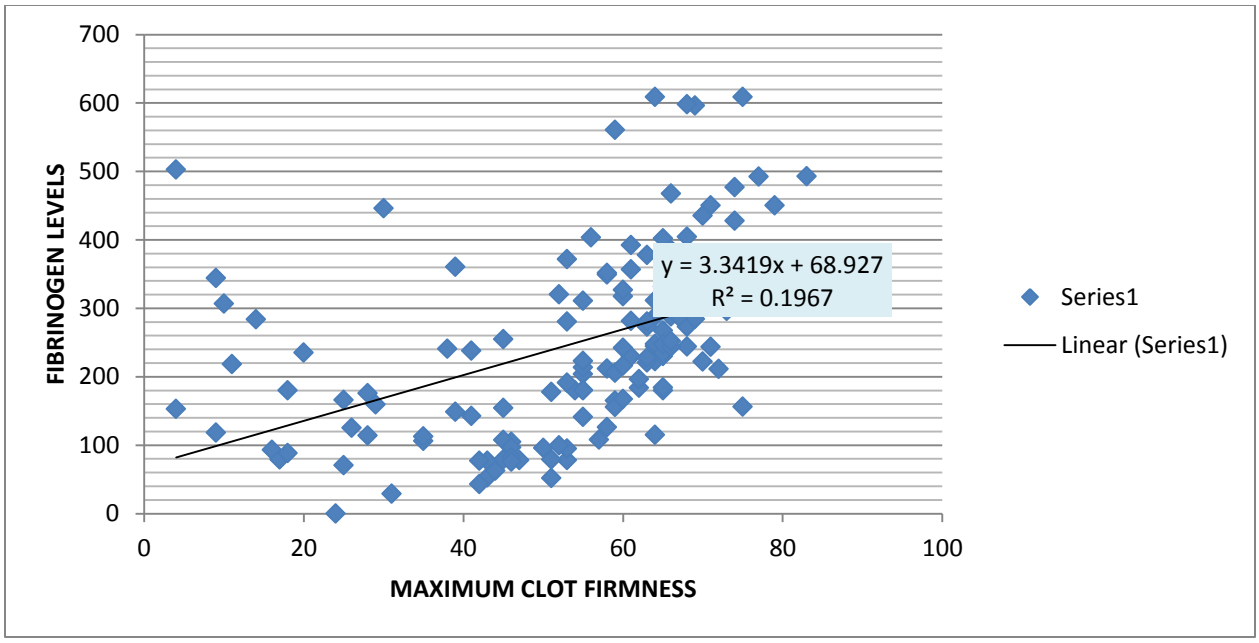

Figure 22 - Scatter diagram of MCF against serum fibrinogen levels.

The Pearson product-moment correlation coefficient (PMCC) that expresses the strength of the linear relationship between Serum fibrinogen and MCF is R= 0.443.

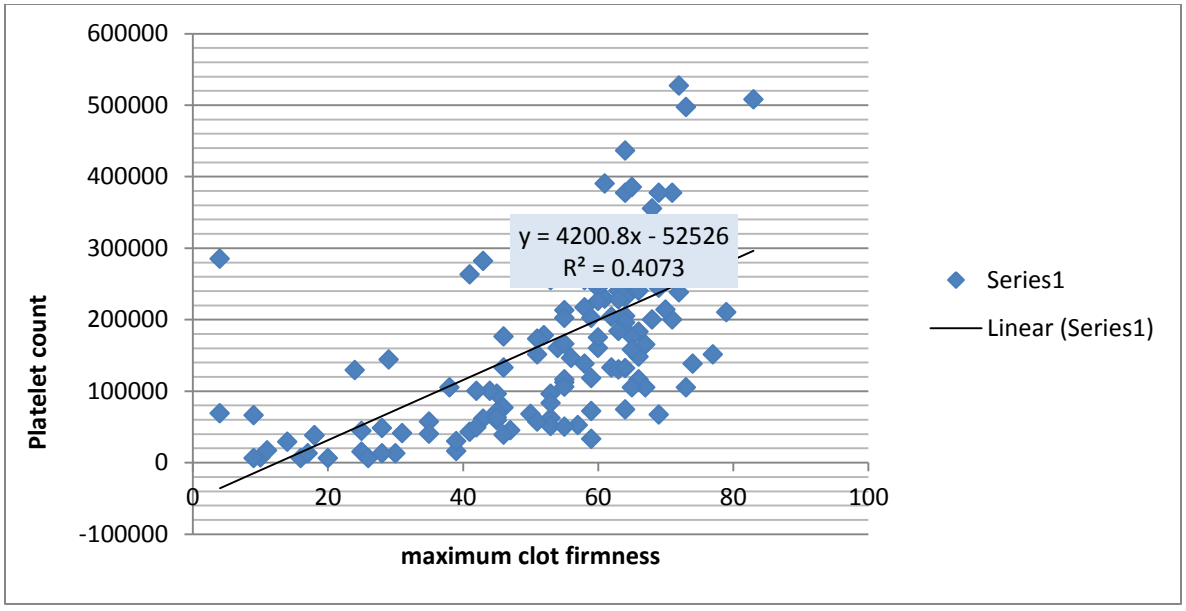

Figure 23- Scatter diagram of MCF against platelet count.

The Pearson product-moment correlation coefficient (PMCC) that expresses the strength of the linear relationship between Platelet count and MCF is R= 0.638

BLOOD PRODUCT	MEAN OF PRODUCT USAGE
RED CELL TRANSFUSION	4.34(0-35)
FFP TRANSFUSION	5.22(0-51)
PLATELET TRANSFUSION	6 (0-77)
CRYOPRECIPITATE TRANSFUSION	9(0-98)

Table 15 - Blood product usage in cases

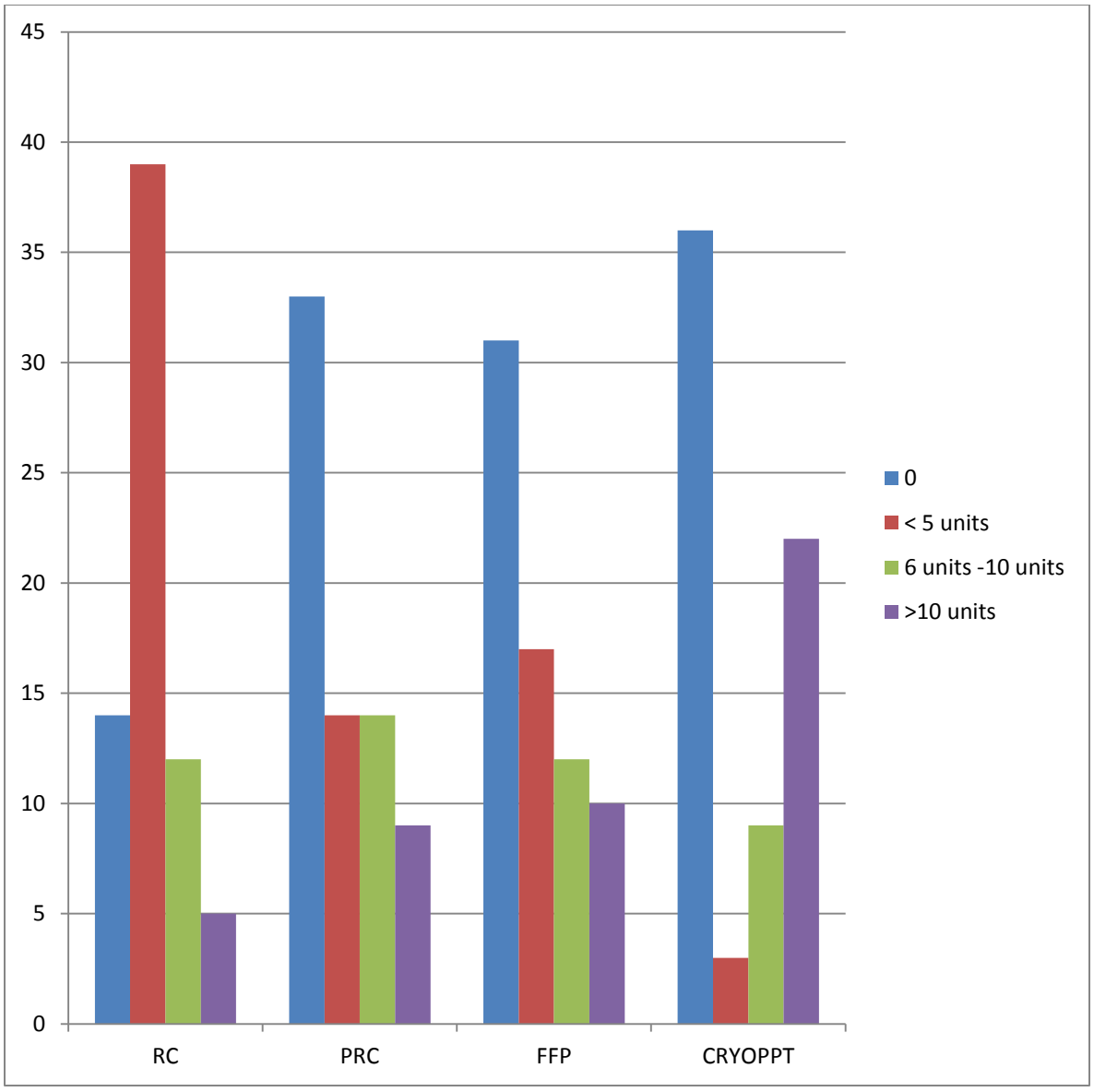

Figure 24 -Use of various blood proucts in cases

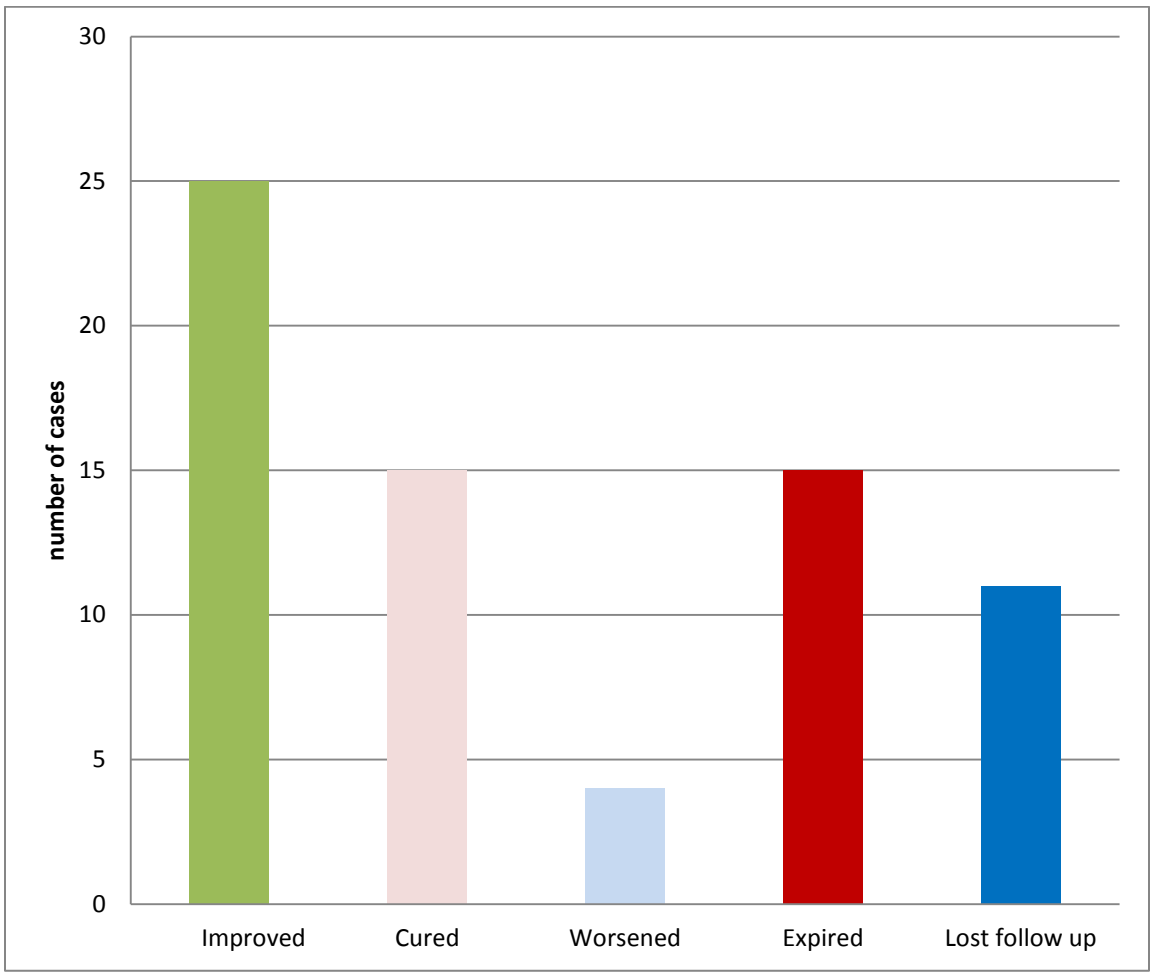

Figure 25 - Frequency of outcome of cases

Comparison of coagulopathy detection by ROTEM- CLOTTING TIME and PROTHROMBIN TIME in Standard coagulation profile		PROTHROMBIN TIME		TOTAL
		POSITIVE	NEGATIVE	
ROTEM CLOTTING TIME	POSITIVE	12	06	18
	NEGATIVE	52	62	114
TOTAL		64	68	132

Table 16 - Comparison of ROTEM - Clotting Time (CT) with the Prothrombin time (PT)

The Kappa Statistic for agreement between ROTEM – CT and PT in detection of coagulopathy is K = 0.101 (-0.019 to 0.221) indicating a POOR agreement between the ROTEM -CT and PT. ($p<0.129$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	←
0.21-0.40	Fair	
0.41-0.60	Moderate	
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 17- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM – Clotting Time in comparison to the Prothrombin Time:

Sensitivity of the ROTEM- CT compared to the PT= a/a+c=12/64

= 18.75% (95% C.I. =10.09 % to 30.47 %)

Specificity of the ROTEM – CT compared to the PT = d/b+d = 62/68

= 91.18% (95% C.I. =81.77 % to 96.67%).

Comparison of coagulopathy detection by ROTEM- CLOTTING TIME and coagulation profile ACTIVATED PARTIAL THROMBOPLASTIN TIME		ACTIVATED PARTIAL THROMBOPLASTIN TIME		TOTAL
		POSITIVE	NEGATIVE	
ROTEM CLOTTING TIME	POSITIVE	12	05	17
	NEGATIVE	42	63	105
TOTAL		54	68	122

Table 18- Comparison of ROTEM Clotting time (CT) with Activated partial thromboplastin time(APTT)

The Kappa Statistic for agreement between CT and APTT in detection of coagulopathy is

K =0.160 (0.023-0.297) indicating a POOR agreement between the CT and APTT. ($p<0.033$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	←
0.21-0.40	Fair	
0.41-0.60	Moderate	
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 19- Interpretation of the Kappa statistic

Analyzing the test characteristics of the CT in comparison to the APTT:

Sensitivity of the CT compared to the APTT = a/a+c =12/54

=22.22% (95% C.I. =12.06-35.60)

Specificity of the CT compared to the APTT = d/b+d = 63/68

=92.65% (95% C.I. =83.66 – 7.54).

Comparison of coagulopathy detection by ROTEM- CFT and SCP-PT		PROTHROMBIN TIME		TOTAL
		POSITIVE	NEGATIVE	
ROTEM CLOT FORMATION TIME	POSITIVE	27	3	30
	NEGATIVE	37	65	102
TOTAL		64	68	132

Table 20- Comparison of ROTEM Clot Formation Time (CFT) with the Prothrombin time(PT)

The Kappa Statistic for agreement between ROTEM CFT and APTT in detection of coagulopathy is K = 0.384 (0.248-0.519) indicating a FAIR agreement between the CFT andAPTT . ($p<0.000$) the sensitvity

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	←
0.41-0.60	Moderate	
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 21- . Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM – CFT in comparison to the APTT:

Sensitivity of the ROTEM- CFT compared to the APTT = a/a+c = 27/64

= 42.19% (95% C.I. =29.94-55.18)

Specificity of the ROTEM- CFT compared to the APTT = d/b+d = 65/68

= 95.59% (95% C.I. =87.63-99.03).

Comparison of coagulopathy detection by ROTEM- CLOT FORMATION TIME and ACTIVATED PARTIAL THROMBOPLASTIN TIME		ACTIVATED PARTIAL THROMBOPLASTIN TIME		TOTAL
		POSITIVE	NEGATIVE	
ROTEM- CLOT FORMATION TIME	POSITIVE	24	3	27
	NEGATIVE	30	65	95
TOTAL		54	68	122

Table 22- Comparison of ROTEM Clot Formation Time with the activated Partial Thromboplastin Time

The Kappa Statistic for agreement between ROTEM CFT and APTT in detection of coagulopathy is K =0.422(0.275-0.570) indicating a MODERATE agreement between the two-cell and three-cell panels. ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	←
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 23- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM- CFT in comparison to the APTT :

Sensitivity of the CFT compared to the APTT= a/a+c =24/54

= 44.44% (95% C.I. =30.92 -58.60.)

Specificity of the CFT compared to the APTT= d/b+d = 65/68

= 95.59.% (95% C.I. =87.63 -99.03).

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Comparison of coagulopathy detection by ROTEM- CLOT FORMATION TIME and PLATELET COUNT -Standard coagulation profile		PLATELET COUNT		TOTAL
		POSITIVE	NEGATIVE	
ROTEM- CLOT FORMATION TIME	POSITIVE	20	3	23
	NEGATIVE	24	65	89
TOTAL		44	68	112

Table 24- Comparison of ROTEM CFT with the PLATELET COUNT

The Kappa Statistic for agreement between ROTEM CFT and PLATELET COUNT in detection of coagulopathy is K = 0.448 (0.268-0.538) indicating a MODERATE agreement between the CFT and PC ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	←
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 25 -Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM CFT in comparison to the PC:

Sensitivity of the ROTEM- CFT compared to the PC = a/a+c =20/44

= 45.5% (95% C.I. =35.4-50.4)

Specificity of the ROTEM CFT compared to the PC = d/b+d = 65/68

= 95.59.% (95% C.I. =87.63-99.03).

Comparison of coagulopathy detection by ROTEM Clot Formation Time and FIBRINOGEN		FIBRINOGEN		TOTAL
		POSITIVE	NEGATIVE	
ROTEM- CFT	POSITIVE	16	3	19
	NEGATIVE	19	65	84
TOTAL		35	68	103

Table 26- Comparison of ROTEM CFT with the FIBRINOGEN.

The Kappa Statistic for agreement between ROTEM CFT and SCP FIBRINOGEN in detection of coagulopathy is K = 0.465 (0.286-0.644) indicating a MODERATE agreement between the CFT AND FIBRINOGEN . ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	←
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 27- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM CFT in comparison to the FIBRINOGEN

Sensitivity of the ROTEM- CFT compared to the FIBRINOGEN = a/a+c =16/35

=45.71% (95% C.I. =28.84-63.35)

Specificity of the ROTEM- CFT compared to the FIBRINOGEN = d/b+d = 65/68

= 95.59% (95% C.I. =87.63-99.03).

Comparison of coagulopathy detection by ROTEM AA and PLATELET COUNT Standard coagulation profile		PLATELET COUNT		TOTAL
		POSITIVE	NEGATIVE	
ROTEM ALPHA ANGLE	POSITIVE	23	1	24
	NEGATIVE	21	67	88
TOTAL		44	68	112

Table 28- Comparison of ROTEM AA with the PC

The Kappa Statistic for agreement between ROTEM AA and PC .in detection of coagulopathy is K = 0.552 (36.69 -67.53) indicating a MODERATE agreement between the two-cell and three-cell panels. ($p<0.000$.)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	←
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 29- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM in comparison to the SCP:

Sensitivity of the ROTEM AA compared to the PC = a/a+c =23/44

=52.27 % (95% C.I. =36.69-67.53)

Specificity of the ROTEM AA compared to the PC = d/b+d = 67/68

= 98.53.% (95% C.I. =92.05-99.75).

Comparison of coagulopathy detection by ROTEM Alpha angle and Fibrinogen		FIBRINOGEN		TOTAL
		POSITIVE	NEGATIVE	
ROTEM ALPHA ANGLE	POSITIVE	13	1	14
	NEGATIVE	22	67	89
TOTAL		35	68	103

Table 30- Comparison of ROTEM AA with the FIBRINOGEN.

The Kappa Statistic for agreement between ROTEM -AA and FIBRINOGEN in detection of coagulopathy is K =0.418(0.242 -0.593) indicating a MODERATE agreement between AA AND FIBRINOGEN . ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	←
0.61-0.80	Good	
0.81-1.00	Very Good	

Table 31- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM AA in comparison to the FIBRINOGEN :

Sensitivity of the ROTEM AA compared to the FIBRINOGEN = a/a+c =13/35

=37.14% (95% C.I. =21.49-55.07%)

Specificity of the ROTEM AA compared to the FIBRINOGEN = d/b+d= 67/68

= 98.53% (95% C.I. =92.05-99.75).

Comparison of coagulopathy detection by ROTEM MCF and PC		PLATELET COUNT		TOTAL
		POSITIVE	NEGATIVE	
ROTEM MAXIMUM CLOT FIRMNESS	POSITIVE	38	5	43
	NEGATIVE	6	63	69
TOTAL		44	68	112

Table 32 - Comparison of ROTEM MCF with the PC

The Kappa Statistic for agreement between ROTEM MCF and PC in detection of coagulopathy is K =0.793 (0.677-0.909) indicating a GOOD agreement between the MCF AND PC. ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	
0.61-0.80	Good	←
0.81-1.00	Very Good	

Table 33- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM MCF in comparison to the Platelet count :

Sensitivity of the ROTEM MCF compared to the Platelet count = a/a+c =38/44

=86.36% (95% C.I. =72.64-94.79)

Specificity of the ROTEM MCF compared to the Platelet count = d/b+d = 63/68

= 92.65% (95% C.I. =83.66-97.54.).

Comparison of coagulopathy detection by ROTEM MCF and FIBRINOGEN		FIBRINOGEN		TOTAL
		POSITIVE	NEGATIVE	
ROTEM MAXIMUM CLOT FIRMNESS	POSITIVE	31	5	36
	NEGATIVE	4	63	67
TOTAL		35	68	103

Table 34- Comparison of ROTEM MCF with the FIBRINOGEN

The Kappa Statistic for agreement between ROTEM MCF and FIBRINOGEN.in detection of coagulopathy is K = 0.807 (0.686 -0.927) indicating a VERY GOOD agreement between the two-cell and three-cell panels. ($p<0.000$)

Value of K	Strength of agreement	Agreement in this study
<0.20	Poor	
0.21-0.40	Fair	
0.41-0.60	Moderate	
0.61-0.80	Good	
0.81-1.00	Very Good	←

Table 35- Interpretation of the Kappa statistic

Analyzing the test characteristics of the ROTEM MCF in comparison to the FIBRINOGEN :

Sensitivity of the ROTEM MCF compared to the FIBRINOGEN = a/a+c =31/35

= 88.57 % (95% C.I. = 73.24-96.73%)

Specificity of the ROTEM MCF compared to the FIBRINOGEN = d/b+d = 63/68

= 92.65% (95% C.I. =83.66-97.54).

ROTEM	COAGULATION PROFILE	K value	Correlation	Sensitivity	Specificity
Clotting Time	Prothrombin Time	0.101	POOR	18.8%	91.2%
Clotting Time	Activated Partial Thromboplastin Time	0.160	POOR	22.2%	92.7%
Clot Formation Time	Prothrombin Time	0.384	FAIR	42.2%	95.6%
Clot Formation Time	Activated Partial Thromboplastin Time	0.422	MODERATE	44.4%	95.6%
Clot Formation Time	Platelets	0.404	MODERATE	45.5%	95.6%
Clot Formation Time	Fibrinogen	0.465	MODERATE	45.7%	95.6%
Alpha Angle	Platelets	0.552	MODERATE	52.3%	98.5%
Alpha Angle	Fibrinogen	0.418	MODERATE	37.1%	98.5%
Maximum Clot Firmness	Platelets	0.793	GOOD	86.3%	92.7%
Maximum Clot Firmness	Fibrinogen	0.807	VERY GOOD	88.6%	92.7%

Table 36- Corelation between Rotem parameters and Standard coagulation profile

DISCUSSION:

The management of acquired bleeding disorders depends on the clinical diagnosis that causes the bleeding disorder. In our institution any patient admitted with traumatic injury, severe blood loss, coagulopathies of any cause are given blood product support. The blood bank doctor or the clinical hematologist is informed regarding patients clinical condition, who suggests the necessary blood product to be transfused. The samples are drawn for preliminary investigations which include the standard coagulation tests and complete blood counts. The transfusion therapy is given empirically, starting with cryoprecipitate followed by other products like red cell concentrate, fresh frozen plasma and platelets. Further transfusion requirements are based on the coagulation parameters of the patient, the results of which take about 45 minutes to be available online. In case of postoperative bleeding in cardio thoracic and liver transplant cases, coagulopathy monitoring is done by standard coagulation tests and thromboelastometry, as per the surgeons request.

Our study aimed to compare the thromboelastometry method to standard coagulation profile in detecting coagulopathies. The current study is a diagnostic study and included 70 patients with acquired bleeding disorders and 68 controls who had normal coagulation profile. In all samples the standard coagulation tests (gold standard) and thromboelastometry were performed.

The role of thromboelastometry in assessing coagulopathy has not been extensively studied in India. There are several observational studies showing poor correlation between Prothrombin Time and activated Partial Thromboplastin Time to Clotting Time , a good correlation between Prothrombin Time and activated Partial Thromboplastin Time to Clot Formation Time, and a very good correlation between Fibrinogen level and Maximum Clot Firmness (132) Platelet count and fibrinogen levels correlated significantly with clot strength, and fibrinogen levels

correlated with fibrin polymerization.(133) A significant correlation was found between activated partial thromboplastin time and Clot Formation Time.

A total of 138 cases were included in this study. The mean age of males in the case group was 44 years and that of female patients were 36 years while in control group was 36 years and 31 years for men and women respectively. The mean age of all patients in the study group was 37 years. The reference ranges for standard coagulation profile and ROTEM variables differ in children and so pediatric bleeding cases were not taken up for the study.

From our study we found that ROTEM MCF and Fibrinogen has a very good agreement K = 0.807 ($p<0.000$) with 88.6% of cases showing a decreased maximum clot firmness. Similar results were obtained by a study done by Tauber et al. (126) We evaluated the cases with fibrinogen levels less than 150 mg/dl as this was considered as a threshold of cryoprecipitate transfusion in our institution. The fibrinogen levels ranged from undetectable levels to 148.9 mg/d. The clinical conditions which led to decreased fibrinogen levels that correlated well with MCF values were coagulopathy due to trauma and obstetric causes. There are several studies on obstetric conditions leading to coagulopathy and diagnosis on the basis of thromboelastometry.(120) The sensitivity and specificity were high, 88.57 % and 92.65% . Severe depletion of plasma fibrinogen levels below 1.0 g /liter were observed in 63%.

ROTEM maximum clot firmness and platelet count has a GOOD agreement K =0.793 (0.677-0.909) indicating a good agreement between the Maximum clot firmness and platelet count ($p<0.000$). The sensitivity was 86.36% and specificity 92.65%. The platelet count of these 44 patients ranged from 6000/cumm to 96,000/cumm. This finding was similar to data from liver

transplantation in adults, where thromboelastometry was used to assess the coagulation status(135) Thus, MCF values might serve as surrogate parameters to estimate platelet function.

There was a moderate response in the following correlations

1. Clot formation time and activated partial thromboplastin time
2. Clot formation time and platelet count
3. Clot formation time and fibrinogen
4. Alpha angle and platelet count
5. Alpha angle and fibrinogen.

Clot formation time and activated partial thromboplastin time

Out of the 54 cases who had a prolonged aPTT , only 24 cases showed prolonged clot formation times giving a sensitivity of 44.44%, while in the control group only three presented with a prolonged clot formation time, thereby giving a specificity of 95.59 %. The kappa statistic for agreement was 0.422, $p<0.000$. This is in contrast to a study by Haas et al and Rugeri et al which showed higher correlation between of activated partial thromboplastin time (aPTT) and CFT (132,135)

Clot formation time and platelet count, fibrinogen

Out of the 44 cases who had a platelet count less than one lakh, only 20 cases had prolonged clot formation times (sensitivity - 45.5%), while in the control group only three presented with a prolonged clot formation time, thereby giving a specificity of 95.6 %. The kappa statistic for agreement was 0.448, $p<0.000$. CFT and fibrinogen gave a moderate correlation and had a

sensitivity of only 45.71% while the specificity was high. There has not been much studies on Clot formation time and platelet count and fibrinogen.

Alpha angle and platelet count

Out of the 44 cases who had a platelet count less than one lakh, only 23 cases had a decreased alpha angle (sensitivity – 52.3%), while in the control group only one presented with a decreased alpha angle, thereby giving a specificity of 98.5 %. The kappa statistic for agreement was 0.552, $p<0.000$. A study by Theusinger et al showed that there is a significant correlation between alpha angle and platelets.(121) Alpha angle takes into account the functional ability of platelets in addition to the platelet count. Therefore in alpha angles will be normal when the counts are low and when the platelet function is good.

Alpha angle and fibrinogen

The alpha angle gave a moderate correlation with the Fibrinogen, $k = 0.418$, while studies have shown that there is a good correlation between alpha angle and fibrinogen.(121)

CT and PT

There is a poor correlation between Clotting Time and Prothrombin Time in this study. This is contradictory to the study by Haas et al where there was good correlation between PT and CT.(132)

CT and APTT

There is a poor correlation between Clotting Time and Prothrombin Time and Clotting Time and APTT. Study by Haas at showed a high correlation to the CFT and aPTT which was contradictory to our findings. (132)

LIMITATIONS

Predictive values could not be ascertained because it is not a prevalence study.

The data was analyzed in the binary or dichotomous mode which could have been processed in the ordinal mode.

We were not able to correlate maximum lysis with any factor, since all patients did not have a D Dimer or factor XIII levels done.

We could only collect the correct sample size for cases with low fibrinogen levels whereas in other cases we had sample size more than that was required for this type of study which was calculated on the basis of literature reviews and pilot studies done earlier in the department.

CONCLUSIONS

The achievement of hemostasis is a crucial factor for determining patient outcomes in acquired bleeding disorders. The standard coagulation tests are still the gold standard for the diagnosis of acquired coagulation factor deficiencies. These tests are designed in such a way that it is incapable of representing the balance of coagulation, as it is an in vitro test. Rotational Thromboelastometry analysis can be performed as bedside tests but standard coagulation profile can be done in a central laboratory and this can cause a delay for sample transport. The turnaround time for standard coagulation tests are long (average- 45 minutes) compared to the earlier results of thromboelastometry which are obtained within ten minutes. Therefore thromboelastometry can be used as a screening method for detecting thrombocytopenia and hypofibrinogenemia in hemorrhagic conditions and can help in early transfusion support of these patients.

ABBREVIATIONS

1. PT- Prothrombin time
2. APTT-Activated Partial Thromboplastin Time
3. INR- International Normalized Ratio
4. PC-Platelet Count
5. CT-Clotting Time
6. CFT-Clot Formation Time
7. AA- Alpha Angle
8. MCF- MaximumClot Firmness
9. ML-Maximum Lysis
10. DIC-Disseminated Intravascular Coagulation
11. TPAI-Tissue Plasminogen Activator Inhibitor
12. SCP-Standard Coagulation Profile

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ANNEXURES

Data entry form

COMPARITIVE EVALUATION OF ROTEM WITH STANDARD COAGULATION PROFILE IN ACQUIRED BLEEDING DISORDER CASES

Department of Transfusion Medicine and Immunohematology
Christian Medical College,Vellore

Name of patient		Unique study no.	
Age/DOB		Telephone no.	
Department/Firm		Address	
Hospital no.			

PATIENT INFORMATION		
CLINICAL FEATURES		DIAGNOSIS
CBC	Hb:	
	Total count:	Differential Count:
	Platelet count:	
COAGULATION PROFILE	PT:	INR:
	APTT:	
	Platelet Count:	
	S.Fibrinogen:	
ROTEM	Clotting Time:	
	Clot formation time:	
	Alpha angle:	
	Maximum clot firmness:	
	Maximum Lysis:	
TRANSFUSION REQUIREMENTS		
TREATMENT OUTCOME		

Institutional Review Board Approval

INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

August 19, 2013

Dr. Soonam John
PG Registrar
Department of Transfusion Medicine and Immunohaematology
Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project:**
Comparative evaluation of rotational thromboelastometry (ROTEM) with the standard coagulation profile in acquired bleeding disorder cases to diagnose coagulation abnormalities.
Dr. Soonam John, PG Registrar, Dr. Sukesh Chandran Nair, Transfusion Medicine and Immunohaematology.

Ref: IRB Min. No. 8333 [DIAGNOSE] dated 18.06.2013

Dear Dr. Soonam John,

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Sukesh Chandran Nair, Transfusion Medicine and Immunohaematology, CMC

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of Rs. 60,000/- (Rupees Sixty Thousand only) for 18months.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Sukesh Chandran Nair, Transfusion Medicine and Immunohaematology, CMC

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Data Excel sheets – Cases, Controls and Codes

SLNO	UIN	AGE	GENDER	BLEEDING DISORDER	MEDICATIONS	DIAGNOSIS	Hb	Platelet Count	PT	PT-C	INR	APTT	APTT-C	FIBRINOGEN	CT	CFT	alpha angle	MCF	ML	RC	PRC	FFP	Cryoppt	OUTCOMES	Date of test	Last date of review
1	B01	62	0	10	0	1	11.2	57,000	16.4	12.1	1.47	60.5	31.3	106.3	276	385	43	35	1	3	8	8	22	1	26.08.2013	09.11.2013
2	B02	41	1	18	0	6	9.3	2,44,000	11.5	0	1.05	72.8	58.2	327	687	110	70	69	14	0	0	0	0	2	20.02.2013	25.02.2013
3	B03	52	0	24	0	2	3.4	2,85,000	120	11.5	10	180	26.9	502.8	1340	4061	8	4	1	5	3	10	0	2	01.06.2013	06.06.2013
4	B04	45	0	15	3	13	10.7	68,000	12.8	11.6	1.16	26.2	0	96	246	152	62	50	13	0	0	0	0	2	02.08.2013	14.08.2013
5	B05	30	0	4	0	4	14.1	2,17,000	10.8	0	0.99	59	30.5	349.1	704	172	58	58	7	0	0	2	0	5	05.08.2013	05.08.2013
6	B06	43	0	9	0	5	4.4	67,000	14	11.8	1.28	29.4	0	596	326	207	54	69	1	4	0	0	0	1	15.03.2013	22.03.2013
7	B07	26	1	10	0	11	7.6	96,000	16.5	11.8	1.48	61.2	28.5	78.2	204	142	67	53	2	7	4	4	12	2	28.08.2013	01.09.2013
8	B08	61	0	10	0	3	7.1	1,12,000	17	11.8	1.53	107.2	30.7	203.8	418	150	61	55	4	4	0	8	6	4	04.09.2013	07.09.2013
9	B09	20	0	24	0	3	3.4	96,000	21	12.1	1.86	93.3	51.4	78.9	552	190	57	45	1	35	13	51	30	4	09.09.2013	12.09.2013
10	B10	58	1	6	0	3	9.9	2,10,000	34.5	12.6	2.99	47.4	27.6	450.4	467	220	56	79	1	2	0	4	0	4	25.09.2013	28.09.2013
11	B11	63	0	19	0	12	5.6	52,000	16.6	12.1	1.49	35.5	0	108.1	295	112	69	57	1	5	0	8	24	1	24.10.2013	26.10.2013
12	B12	22	1	20	0	5	11.1	15,000	12.4	0	1.12	48.4	28.3	165.9	468	1231	19	25	2	7	46	16	79	1	03.08.2014	30.08.2014
13	B13	45	0	10	4	1	13.6	77,000	16.6	12.2	1.52	61.4	32.4	104.6	237	195	58	46	7	1	0	9	16	1	26.06.2014	15.07.2014
14	B14	52	1	11	0	6	11.5	4,36,000	16.1	11.4	1.43	43.3	30.7	222.4	403	116	67	64	11	0	0	0	0	5	12.07.2013	12.07.2013
15	B15	18	0	2	0	10	4	69,000	14.8	11.8	1.32	49.4	35.2	152.7	570	1188	26	4	1	0	0	0	0	5	14.12.2013	14.12.2013
16	B16	45	0	2	0	5	10.5	38,000	14.5	12.1	1.3	28.4	0	180.2	629	390	10	18	4	10	28	16	6	1	18.01.2014	12.02.2014
17	B17	58	1	21	0	7	12.2	71,000	14.6	11.4	1.3	47.7	31.3	107.4	459	187	57	45	12	0	0	0	0	5	06.01.2014	06.01.2014
18	B18	23	0	1	3	10	12.4	2,82,000	15.2	11.7	1.39	39.1	29.5	77.1	342	252	54	43	8	1	0	4	5	3	14.02.2014	23.02.2014
19	B19	54	0	6	0	5	8.2	33,000	14.2	11.5	1.27	43.5	32.4	560.5	473	106	70	59	34	0	0	0	0	5	09.01.2014	10.01.2014
20	B20	32	0	10	4	3	7.7	1,16,000	18	12.2	1.64	49.7	34.2	213.7	493	112	67	55	7	4	0	4	7	1	14.03.2014	05.05.2014
21	B21	19	0	3	0	5	7	6,000	22.5	12.35	2.06	48.9	28.6	92.9	593	202	19	16	5	0	2	4	6	1	24.03.2014	10.04.2014
22	B22	25	1	16	3	5	8.7	62,000	13.3	11.8	1.21	27.3	0	280.6	412	168	58	53	5	3	6	0	0	1	01.04.2014	04.04.2014
23	B23	40	0	22	0	5	6	7,000	12.1	0	1.1	42.1	25.1	306.9	768	220	21	10	2	13	77	24	0	4	04.04.2014	19.04.2014
24	B24	61	1	16	3	5	5.4	17,000	15	12	1.37	23.9	0	218.2	513	186	16	11	7	3	5	22	5	4	05.04.2014	10.04.2014
25	B25	31	1	9	0	3	7.1	3,77,000	16.9	11.9	1.54	88.3	39.6	243.4	531	115	69	71	4	2	0	0	0	1	07.04.2014	08.04.2014
26	B26	68	0	1	0	4	10.7	29,000	12.2	0	1.11	133.3	38.8	284	2209	3	8	14	1	0	0	0	0	5	11.04.2014	11.04.2014
27	B27	37	0	12	3	3	9.6	1,51,000	15.9	12.1	1.45	58.7	29.2	52.1	232	237	50	51	1	2	2	4	0	2	23.04.2014	05.05.2014
28	B28	43	1	3	0	5	9.7	59,000	13	11.8	1.18	28.6	0	254.9	435	232	50	45	4	3	6	20	23	1	23.04.2014	09.05.2014
29	B29	52	0	10	3	3	8.4	72,000	14.2	11.6	1.29	41.4	26	165.2	331	111	68	59	8	12	3	0	6	2	03.05.2014	29.05.2014
30	B30	32	1	23	0	5	3.3	6,000	13.8	0	1.26	47.4	24.4	235.4	328	2403	41	20	6	0	0	0	0	5	31.05.2014	31.05.2014
31	B31	30	0	7	0	10	18.5	13,000	58.6	13.4	5.42	112.3	26.6	79.2	920	3600	14	17	4	0	0	0	0	1	12.05.2014	23.05.2014
32	B32	40	0	10	0	3	8.7	74,000	13.4	11.5	1.22	42.4	28.6	608.8	303	102	69	64	3	6	8	8	6	1	22.05.2014	17.06.2014
33	B33	36	0	15	0	3	5.4	45,000	16.7	11.6	1.52	44.1	29.2	78.1	237	183	60	47	3	8	8	0	12	4	12.06.2014	15.06.2014
34	B34	22	0	10	0	11	10.6	39,000	14.1	11.5	1.29	28.9	0	96.9	230	210	60	46	4	3	0	6	0	2	12.06.2014	25.06.2014
35	B35	28	1	10	0	11	4.9	61,000	28.7	13.7	2.63	76.7	32.4	52.9	367	213	62	43	2	8	8	6	20	2	14.03.2014	15.03.2014
36	B36	47	0	2	0	8	14.9	2,05,000	17.5	12	1.6	34.3	0	287.7	342	103	69	64	2	1	0	0	0	1	02.06.2014	03.06.2014
37	B37	50	0	15	0	3	8.5	2,60,000	13.5	11.5	1.23	31.1	0	126.4	223	107	69	58	6	4	0	0	0	2	03.06.2014	11.06.2014
38	B38	34	0	17	3	3	8.1	1,33,000	18.7	12.5	1.71	42.3	30.3	82.2	255	195	58	46	7	2	4	4	18	1	23.06.2014	20.07.2014
39	B39	46	0	15	0	5	6.6	44,000	36.6	12.4	3.37	42.4	27.4	70.8	465	978	34	25	10	30	20	10	98	4	04.07.2014	06.07.2014
40	B40	26	1	12	0	11	9.2	1,76,000	15.2	12.4	1.39	37.2	31.8	75.7	310	253	49	46	5	2	4	0	6	2	26.06.2014	28.06.2014
41	B41	68	0	15	4	9	5.7	43,000	14	11.5	1.28	46.5	34.7	142.5	276	246	53	41	9	3	6	4	6	1	03.07.2014	23.07.2014
42	B42	69	0	9	4	10	5.6	16,000	15.7	11.7	1.41	32.2	0	360.4	661	422	41	39	2	6	10	4	0	1	02.06.2014	18.06.2014
43	B43	62	1	10	4	3	11.9	51,000	16.6	12.5	1.52	48.2	29.1	94.9	282	142	64	53	3	4	6	0	12	1	06.05.2014	29.05.2014
44	B44	22	1	12	0	11	5.9	49,000	20.2	12	1.79	71.4	32.5	77.1	413	373	38	42	2	7	33	23	32	4	19.07.2014	22.07.2014
45	B45	19	1	15	4	3	8.6	83,000	18.9	11.4	1.73	31.4	0	371.9	199	144	63	53	14	5	0	0	0	3	08.07.2014	09.07.2014
46	B46	46	0	15	0	3	5.3	1,00,000	17.2	11.7	1.57	45.6	32.5	62.7	234	195	58	44	13	9	2	12	20	5	17.06.2014	20.06.2014
47	B47	29	1	16	0	8	8.3	6000	14.4	10.4	1.31	34.5	0	118.1	684	218	32	9	11	8	12	0	12	4	17.06.2014	18.06.2014
48	B48	55	0	10	0	3	8.7	50,000	24.8	13.4	2.27	39.7	32.5	311	343	226	51	55	1	3	4	0	0	5	30.06.2014	05.07.2014
49	B49	29	1	12	0	11	5.5	41,000	19.3	11.3	1.72	38	29.4	28.9	456	771	26	31	1	4	10	4	6	2	20.07.2014	25.07.2014
50	B50	73	0	8	0	10	14.3	2,63,000	24.6	12.2	2.25	56.3	35.1	238	1023	719	21	41	1	0	0	0	0	4	30.06.2014	01.07.2014
51	B51	28	1	15	0	3	8.3	1,46,000	14.4	11.3	1.3	30.8	0	403.7	245	122	66	56	14	2	0	0	0	1	22.07.2014	02.08.2014
52	B52	64	0	10	3	3	8.1	3,77,000	14.5	11.4	1.32	47.7	27.6	115	202	79	74	64	1	4	0	4	0	3	04.06.2014	20.06.2014
53	B53	56	0	15	0	3	9.2	66,000	13.7	10.9	1.25	40.4	29.6	344.3	394	186	44	9	1	11	8	4	24	3	04.06.2014	12.07.2014
54	B54	55	0	10	0	1	6.8	40,000	19.2	12.6	1.71	54.5	33.3	112.9	325	439	41	35	3	4	23	12	24	1	09.10.2013	26.10.2013
55	B55	28	0	10	0	3	9.6	48,000	17.2	12.4	1.52	56	30.2	114	356	336	60	28	5	2	8	0	0	5	03.06.2013	06.06.2013
56	B56	56	0	10	3	1	7.6	57,000	19.3	12.2	1.7	55.9	31.6	79.2	341	172	59	51	2	0	0	0	16	1	16.01.2014	03.02.2014
57	B57	58	0	10	3	1	5.9	1,00,000	27.2	12.9	2.34	79.2	30.8	43	225	271	50	42	2	3	4	0	4	1	24.11.2013	23.12.2013
58	B58	40	0	16	0	5	9.6	6,000	13.6	12	1.24	25	0	125.1	1708	423	32	26	5	0	0	0	0	5	30.05.2014	30.05.2014
59	B59	36	1	10	0	3	12.4	1,78,000	14.5	11.4	1.32	28.5	0	99.5	189	148	64	52	6	4	0	0	0	2	12.06.2014	28.06.2014
60	B60	35	1	24	0	13	8	13,000	17.5	11.4	1.6	42.9	31.2	446	520	708	38	30	2	2	8	4	0	4	10.07.2014	12.07.2014
61	B61	20	0	15	0	12	12.2	38,000	15.1	11.3	1.36	99.9	34.6	88.6	845	726	20	18	3	2	3	2	12	2	11.08.2014	12.08.2014
62	B62	38	0	15	0	3	7.2	1,33,000	18.4	12.6	1.64	40.2	32.8	183.9	318	85	73	62	3	6	10	8	12	1	05.08.2014	21.08.2014
63	B63	19	0	15	0	12	12.7	1,44,000	18.4	11.4	1.64	56.3	34.1	159.3	1078	745	23	29	2	2	0	0	0	4	02.08.2014	05.08.2014
64	B64	25	0	6	0	10	14.1	1,29,000	120	12.7	10	175	26.2	0	421	1251	30	24	2	2	0	0	0	4	30.07.2014	03.08.2014
65	B65	18	0	24	0	12	13	13,000	12.6	0	1.17	54.7	36.8	176	650	793	29	28	3	2	4	4	0	2	30.07.2014	05.08.2014
66	B66	55	1	15	0	12	7.2	138000	15.7	12.6	1.41	40.3	30.3	212	406	90	72	58	10	4	2	6	0	4	30.07.2014	17.08.2014
67	B67	73	0	10	4	3	5.1	3,02,000	13.5	11.3	1.22	49.1	28.6	222.1	269	70	76	70	3	2	0	0	0	4	25.07.2014	25.07.2014
68	B68	18	1	13	0	11	12.3	63,000	11.9	0	1.08	40	28.9	154.5	265	200	58	45	9	2	0	3	0	2	12.07.2014	25.07.2014
69	B69	57	0	10	1	10	7.4	106000	14.7	11.2	1.32	37.2	28.3	141.2	169	85	73	55	3	2	0	8	12	1	23.08.2014	30.08.2014
70	B70	21	1	10	0	11	3.8	30000	15.5	11.9	1.39	44.2	22.4	148.9	291	343	42	39	4	9	24	12	30	1	30.08.2014	01.09.2014

SL NO	UIN	AGE	GENDER	BLEEDING DISORDER	MEDICATIONS	DIAGNOSIS	Hb	Platelet Count	PT	INR	APTT	FIBRINOGEN	CT	CFT	alpha angle	MCF	ML
1	A01	65	1	2	0	0	13.4	5,27,000	10.9	1	28.1	358.3	251	52	79	72	10
2	A02	30	1	3	0	0	11.5	3,13,000	12.4	1.13	31.3	229.5	286	84	73	61	18
3	A03	28	1	2	0	0	13.3	1,96,000	11.9	1.09	26.1	244.6	311	87	73	64	17
4	A04	29	1	0	0	0	15.3	2,55,000	109	1	29.3	351.7	308	96	74	58	18
5	A05	36	1	1	0	0	14.2	2,29,000	12	1.1	29.8	392.3	446	136	64	61	17
6	A06	33	1	1	0	0	13.8	2,76,000	11.4	1.04	32.7	365.1	287	72	75	68	14
7	A07	76	0	8	0	0	10.8	1,16,000	11.8	1.08	27.9	385.2	265	75	75	66	13
8	A08	51	1	1	0	0	12.5	1,83,000	10.3	0.95	27.9	467.8	132	150	75	66	10
9	A09	28	1	11	0	0	13.5	2,31,000	10.8	0.99	33.4	311.4	239	79	74	64	18
10	A10	18	0	5	0	0	14.7	3,85,000	11.4	1.07	29.1	230.1	251	80	79	65	14
11	A11	61	0	8	0	0	10.9	1,05,000	10.7	0.97	31.7	339.2	559	126	66	67	7
12	A12	18	0	0	0	0	14	2,04,000	11.4	1.04	29.5	196.5	276	110	68	62	14
13	A13	42	1	11	0	0	10.5	1,38,000	10.5	0.96	27.7	428	284	81	76	74	7
14	A14	29	1	1	0	0	8.7	1,30,000	10.9	1	30.2	220.9	327	82	74	63	7
15	A15	22	1	11	0	0	12.5	1,76,000	10.9	1	26.3	267.6	382	123	67	65	2
16	A16	59	0	0	0	0	8.5	1,65,000	12.3	1.12	28.3	349.1	302	98	71	67	9
17	A17	21	0	0	0	0	16.6	2,13,000	11.5	1.04	33.4	180	325	138	65	55	9
18	A18	30	0	10	0	0	15.3	1,73,000	11.4	1.04	29.3	177.8	383	194	55	51	2
19	A19	20	1	5	0	0	6.8	5,08,000	11.9	1.09	29.2	492.7	279	70	79	83	6
20	A20	24	1	4	0	0	10.9	2,80,000	11.4	0.99	34.7	180	309	87	74	65	15
21	A21	18	1	11	0	0	12.8	2,50,000	10.7	0.98	34.2	301.9	365	89	73	65	6
22	A22	28	0	2	0	0	14.1	3,90,000	11.5	1.05	30.2	356.9	382	112	68	61	8
23	A23	25	1	0	0	0	12.9	2,44,000	11.1	1.02	30.4	317.4	372	126	65	60	10
24	A24	30	1	11	0	0	13.9	2,26,000	11.4	1.04	30.4	327	245	92	72	60	16
25	A25	53	1	10	0	0	12.9	1,84,000	10.1	0.93	25	377.9	267	88	74	63	13
26	A26	38	1	13	0	0	8.6	1,48,000	11.8	1.08	30	288.2	316	114	68	66	0
27	A27	53	0	2	0	0	13.9	1,05,000	10.7	0.98	35.3	365	198	181	65	73	4
28	A28	24	1	11	0	0	10	2,94,000	12.5	1.14	33	402	126	65	65	65	3
29	A29	65	0	3	0	0	13.5	2,59,000	11.2	1.03	30.6	320.2	350	178	60	52	5
30	A30	35	0	1	0	0	16.5	1,32,000	10.7	0.98	33.3	248	292	118	67	64	3
31	A31	47	0	11	0	0	10.7	3,12,000	10.8	0.99	31.7	295.3	294	90	74	68	11
32	A32	27	0	11	0	0	10.5	2,00,000	10	0.97	24	450.4	287	73	76	71	2
33	A33	25	1	3	0	0	8.4	1,51,000	10.5	0.97	29.1	492.3	319	100	73	77	6
34	A34	31	1	3	0	0	9.7	2,14,000	10.3	0.95	24	435.3	319	96	72	70	11
35	A35	24	0	10	0	0	10.8	2,78,000	11.5	1.05	29.8	156	479	104	73	75	7
36	A36	40	0	10	0	0	11.8	1,60,000	11.2	1.03	25	242	264	269	51	60	4
37	A37	47	0	0	0	0	10.5	2,51,000	12.5	1.14	30.3	332.1	442	94	71	65	11
38	A38	37	1	3	0	0	12.1	294000	11.5	1.05	31.1	404.5	367	96	72	68	9
39	A39	39	1	11	0	0	8.9	1,18,000	11.7	1.07	29.7	205.7	626	275	57	59	0
40	A40	53	0	0	0	0	13.4	2,74,000	11.2	1.03	30.4	476.9	302	76	76	74	9
41	A41	29	1	15	0	0	10.6	2,40,000	11.3	1.03	28.5	273.2	260	101	70	63	10
42	A42	23	1	11	0	0	12.6	3,55,000	10.7	0.98	36.6	273.2	355	82	73	68	10
43	A43	22	1	11	0	0	11.2	2,90,000	10.9	0.99	32.6	234.1	341	95	71	65	3
44	A44	22	0	0	0	0	13.7	2,02,000	11.1	1.01	33.6	155.9	432	150	62	59	2
45	A45	25	0	2	0	0	15.5	2,57,000	10.3	0.95	33.5	281.4	396	169	59	61	3
46	A46	32	1	2	0	0	13.1	2,77,000	10.9	0.99	35	371.5	354	82	74	69	9
47	A47	36	0	10	0	0	14.6	2,00,000	10.9	0.99	32.5	243.9	370	107	70	68	5
48	A48	25	1	15	0	0	13.4	2,87,000	10.8	0.98	29	303.4	341	82	74	68	7
49	A49	30	0	7	0	0	14	1,58,000	11.4	1.03	32.2	247.3	347	103	70	65	6
50	A50	31	0	11	0	0	12	1,75,00	10.5	0.95	25.3	215.9	400	138	64	60	12
51	A51	31	0	0	0	0	12.9	2,02,000	11.2	1.02	33.5	222.9	630	167	59	55	11
52	A52	45	1	1	0	0	11.6	2,38,000	9.8	0.89	32.3	211.5	271	81	75	72	8
53	A53	24	1	2	0	0	11.7	2,99,000	11.4	1.04	26	184.2	416	130	65	65	3
54	A54	38	0	0	0	0	22.9	1,05,000	12.2	1.11	31.5	240.7	1093	523	28	38	2
55	A55	67	1	10	0	0	13.2	2,28,000	11	1	27.6	227.8	189	79	74	63	10
56	A56	24	0	15	0	0	14.6	2,57,000	12.4	1.12	36.3	598	736	163	62	68	8
57	A57	23	0	0	0	0	14.8	1,60,000	11.4	1.03	33.5	180.2	589	191	56	54	5
58	A58	30	0	0	0	0	9.7	3,77,000	11.4	1.04	27.3	284.1	321	76	75	69	9
59	A59	30	1	15	0	0	8.2	3,33,000	11	1	30.1	608.9	266	55	79	75	7
60	A60	41	0	0	0	0	15.3	2,70,000	10.5	0.95	30.2	287.7	404	121	68	67	5
61	A61	19	1	11	0	0	10.6	2,40,000	10.5	0.95	29.2	243.4	389	124	65	66	6
62	A62	18	1	11	0	0	12.2	3,19,000	10.9	0.99	33.1	280.6	387	129	66	63	5
63	A63	19	0	5	0	0	14	3,07,000	10.8	0.98	27.7	251.9	321	94	71	66	8
64	A64	25	1	11	0	0	12	1,05,000	11.4	1.04	29.8	320	285	96	71	65	11
65	A65	37	0	2	0	0	7.4	1,66,000	11.8	1.07	32.5	180.7	295	124	66	55	8
66	A66	18	1	8	0	0	11.2	4,97,000	11.1	1.01	30.4	296	260	68	77	73	8
67	A67	20	0	0	0	0	15.7	2,55,000	11.4	1.04	29.1	191.4	379	131	64	53	11
68	A68	19	1	11	0	0	9.6	2,52,000	11.4	1.03	28.5	167.3	409	125	65	60	9

GENDE	BLEEDING DISORDER	MEDICATIONS	DIAGNOSIS
0-Male	0- No bleed	0- Nil	0- NORMAL
1-Female	1- Skin bleeds	1- Antiplatelet	1- ORTHOPTIC LIVER TRANSPLANATION
	2- Epistaxis	2- VKA	2- VIT K DEPENDANT FACTOR DEFICIENCY
	3- Gum bleed	3- Heparin	3- TRAUMA INDUCED COAGULOPATHY
	4- tooth extraction bleeds	4- 1 and 3	4- ACQUIRED F VIII DEF
	5- prolonged bleeds		5- MALIGNANCY ASSOCIATED COAGULOPATHY
	6-haematemesis		6- LUPUS ANTICOAGULANT
	7- Hematochezia		7- SUB ACUTE DIC
	8- Hematuria		8- FACTOR DEFICENCY
	9-Malena		9- SEPSIS RELATED COAGULOPATHY
	10-Intra op bleed		10- COAGULOPATHY ASSOCIATED WITH LIVER DISEASE
	11- Menorrhagia		11- COAGULOPATHY ASSOCIATED WITH OBSTETRIC CASES
	12- PPH		12- SEPSIS ASSOCIATED COAGULOPATHY
	13-Haematuria		13- COAGULOPATHY ASSOCIATED WITH SNAKE BITE
	14- Haemarthrosis		
	15-Others		
	16- IC/SD BLEED		
	17- traumatic bleeds		
	18-intra op+ others		
	19-Hematuria + malena		
	20- skin bleed + gum bleed		
	21- epistaxis + gumbleed		
	22-malena + gumbleed		
	23- gum bleed + menorrhagia		
	24 - multiple bleeds		